Preliminary study of the colour, phenolic and sensory characteristics of Alvarinho and Touriga Nacional wines produced in different Brazilian regions

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Abstract

Over the years, viticulture has expanded to new regions outside the temperate zones, such as in tropical countries, like Brazil. It is important for the productive sector to understand the effects of grapevine interaction with the characteristic of each new region on wines composition. The objective of the study was to evaluate the adaptability of two Portuguese varieties, Alvarinho and Touriga Nacional grown in three Brazilian regions, Rio Grande do Sul, Santa Catarina and Paraná State. The analyses were made starting from samples obtained from the same vintage conducted in 2022 to highlight the differences in the phenolic compounds, (anthocyanins, flavonoids and non-flavonoids, proanthocyanidins and their monomeric, oligomeric and polymeric fractions). The colour parameters and the sensory profile were characterized as well.

During the study, a trend towards higher concentrations in most for the phenolic compounds analysed was observed in the wine samples of Touriga Nacional from Dão Region. Regarding the sensory analysis, the colour intensity of the reds, the most important positive aroma attributes, and the taste balance obtained highest scores in wines from Portugal vineyards. The results suggest that wine samples from Portugal, showed better results in the overall analysis than the samples obtained from Brazilian crops, which were influenced by surrounding environmental conditions less favorable to the development of phenolic compounds in the grapes, having a direct effect also in the analysed wines.

Based on this study, it is envisaged that future research will be conducted with a view to analyzing other chemical compounds in wines that could be influenced by the different terroir; moreover, the prospects are to study new vineyard management techniques aimed at improving the adaptability of non-native varieties to new environmental conditions, or at new winemaking technologies aimed at improving the quality of the wine obtained from these Portuguese varieties in Brazilian regions.

Keywords: *Touriga Nacional, Alvarinho, Brazilian regions, phenolic composition, sensory analysis*

Resumo

Ao longo dos anos, a viticultura se expandiu para novas regiões fora das zonas temperadas, como em países tropicais, como o Brasil. É importante para o setor produtivo entender os efeitos da interação da videira com as características de cada nova região na composição dos vinhos. O objetivo do estudo foi avaliar a adaptabilidade de duas variedades portuguesas, Alvarinho e Touriga Nacional, cultivadas em três regiões brasileiras, Rio Grande do Sul, Santa Catarina e Paraná. As análises foram feitas a partir de amostras obtidas da mesma safra realizada em 2022 para destacar as diferenças nos compostos fenólicos, (antocianinas, flavonoides e não flavonoides, proantocianidinas e suas frações monoméricas, oligoméricas e poliméricas). Os parâmetros de cor e o perfil sensorial também foram caracterizados.

Durante o estudo, observou-se uma tendência para maiores concentrações na maioria dos compostos fenólicos analisados nas amostras de vinho Touriga Nacional da Região do Dão. Relativamente à análise sensorial, a intensidade da cor, os atributos positivos de aroma mais importantes e o equilíbrio gustativo obtiveram pontuações mais elevadas nos vinhos provenientes de vinhas de Portugal. Os resultados sugerem que as amostras de vinho de Portugal, apresentaram melhores resultados na análise global do que as amostras obtidas de colheitas brasileiras, que foram influenciadas por condições ambientais circundantes menos favoráveis ao desenvolvimento de compostos fenólicos nas uvas, tendo um efeito direto também no vinhos analisados.

Com base neste estudo, prevê-se que sejam realizadas pesquisas futuras com vista à análise de outros compostos químicos presentes nos vinhos que possam ser influenciados pelos diferentes terroir; além disso, perspetiva-se o estudo de novas técnicas de gestão da vinha destinadas a melhorar a adaptabilidade das castas não autóctones às novas condições ambientais, ou de novas tecnologias de vinificação destinadas a melhorar a qualidade do vinho obtido a partir destas castas portuguesas nas regiões brasileiras.

Palavras-chave: *Touriga Nacional, Alvarinho, vitivinicultura brasileira, composição fenólica, análise sensorial*

Resumo Alargado

A introdução de castas provenientes de países vitícolas de clima temperado em novas regiões de clima tropical representa um desafio: importa avaliar a adaptabilidade destas castas em novos “terroirs”, não só em termos de produtividade, mas também em termos de composição das uvas e vinho. Considerando a importância da difusão da variedas autóctones portuguesas em muitas regiões do mundo, bastante dispares em termos de condições pedoclimáticas.

Este estudo teve como objetivo apresentar um conjunto de dados relativos aos compostos polifenólicos de duas castas de origem portuguesa cultivadas no Brasil, para avaliar a sua adaptação a diferentes climas e o impacto no vinho. No presente estudo, foram analisadas amostras de vinhos provenientes de duas variedades nativas de Portugal, Alvarinho e Touriga Nacional produsidas em cinco regiões diferentes (Região do Dão, Região dos Vinhos Verdes, Rio Grande do Sul, Santa Catarina e Estado do Paraná).

A Touriga Nacional é uma das principais castas nativas de Portugal, caracterizada por elevado vigor, baixa produção, bagos escuros e pequenos, caracterizada por uma elevada concentração de compostos polifenólicos, sendo uma casta com intensos aromas frutados e florais. O Alvarinho é proveniente da Galiza ou do norte do Minho (Monção e Melgaço): tem uma produção média-alta, vigor médio e cor amarela intensa.

Todas as amostras foram analisadas laboriatamente seguindo métodos científicos padronizados para a determinação dos seguintes parâmetros: fenóis totais, flavonoides, não flavonoides (Kramling e Singleton, 1969), antocianinas e pigmentos corantes (Somers e Evans, 1997), poder tanante (De Freitas e Mateus, 2001), proantocianidinas e suas respectivas frações monoméricas, oligoméricas e poliméricas (Sun *et al*., 1998). As amostras de Touriga Nacional foram ainda submetidas a uma avaliação de cor por CIE/Lab ((Método OIV-MAAS2-11(OIV 2006)) e a uma análise sensorial efetuada por um painel de provadores.

Foram obtidos resultados muito semelhantes entre os vinhos brancos da casta Alvarinho produzidos em Portugal comparativamente a os do Brasil. Para além disso, surgiu uma diferença notável na concentração de compostos fenólicos e cor nas amostras de vinho tinto da casta Touriga Nacional proveniente de Portugal comparativamente a os vinhos da mesma casta, mas produzido nos estades do Rio Grande do Sul e do Santa Catarina.

As regiões brasileiras consideradas no estudo são caracterizados por um clima mais quente e húmido. Estas condições influenciam a síntese de compostos fenólicos durante o amadurecimento da uva ou promovem a sua degradação. Para confirmar a diferença entre as amostras portuguesas e brasileiras, o painel de onze provadores manifestou também uma maior apreciação global, visual, olfativa e gustativa, dos vinhos obtidos a partir da Touriga Nacional cultivada em Portugal. Os atributos considerados foram a intensidade da cor, limpidez, intensidade, persistência, qualidade aromática, aromas (frutados, florais, vegetais e de carvalho), acidez, doçura, amargor, persistência na boca, adstringência e equilíbrio gustativo.

Com a este estudo preliminar, foi possível analisar a potencial adaptabilidade das duas castas Poruguesas estudadas em diferentes condicoes edafoclimaticas do Sul do Brasil. Nesto caso, este adaptalibidade potencial foi analisada em termos das caracteristicas quimicas e sensoriais dos vinhos, a partir das castas Portuguesas.

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Abbreviations

TN = Touriga Nacional

AL = Alvarinho

PT = Portugal

RG = Rio Grande do Sul

SC = Santa Catarina

PR = Paranà State

TNPT = Touriga Nacional cultivated in Portugal

TNRG = Touriga Nacional cultivated in Rio grande do Sul

TNSC = Touriga Nacional cultivated in Santa Catarina

ALPT = Alvarinho cultivated in Portugal

ALRG = Alvarinho cultivated in Rio Grande do Sul

ALPR = Alvarinho cultivated in Paraná State

Mv = Microvinification

Av = Average

St. Dev. = Standard Deviation

L* = Clarity

a* = Red – green colour

b* = Yellow – blue colour

C* = Chroma

H* = Hue

H* C = H*correction

C.I. = Colour Intensity

TO = Tonality

T.A. = Total Anthocyanins

I.D. = Degree of Ionization

I.A. = Ionized Anthocyanins

P.I. = Polymerization Index

T.P. = Total Pigments

P.P. = Polymerized Pigments

BSA = Bovine Serum Albumin

1.Introduction

Over the past two decades, several international grape varieties from different traditional wine- growing countries such as France, Portugal, Italy and Spain have been introduced into different wine-growing regions of the world. The introduction of new vines in emerging regions with environmental conditions different from those of the original production area, issues new challenges on the adaptability of new varieties to new specific "terroirs", in terms of productivity but also in relation to the characteristics of the grape and of the chemical composition of wine (Otto *et al*., 2022). With regard to this important issue, numerous studies have been conducted on the adaptation of different vines to different environments and the impact on their qualitative characteristics. Many studies have been done on international grape varieties, and according to Anderson and Aryal *et al*., (2013), Cabernet and Merlot grape varieties have more than doubled their vineyard area, and based on these, it has been shown that the composition of grapes and wine obtained from different grape varieties depends on various factors that change according to the intrinsic potential of each vine but also according to climatic factors, such as exposure to sunlight, solar radiation, temperature, soil, agricultural practices and also the level of ripeness of the soil.

The terroir includes a set of specific characteristics of the soil, topography, climate and biodiversity, so it is possible to consider that the adaptability of varieties grown outside their region of origin, depends on a series of factors that influence then on the quality of the wine produced. Some studies have been conducted by O-Marques *et al*., (2005) and by Cosme *et al*., (2009) based on the comparison of native French ripening vines introduced in Portuguese territory, and in terms of concentration of phenolic compounds in relation to the climatic conditions and temperature of the day and of the night. Some vines such as Syrah, Cabernet Sauvignon and Alicante Bouchet have lower thermal requirements to reach normal ripeness than some native vines of Portugal, so they may have a different sugar content and titratable acidity in some regions of Portugal with high temperatures compared to the three areas from France.

According to Costa *et al*., (2015) the genetic factor has an important role on the phenolic content, sometimes more than the climatic conditions; according to Flamini *et al*., (2013) and Sikuten *et al*., (2021), the composition of individual anthocyanins is under genetic control, while agronomic practices and climatic conditions have a greater impact on the total content. To date many varieties have been introduced in different geographical

locations, such as South America, Argentina, Chile and Brazil; in recent years several studies have been published showing the results on the adaptability of the various varieties in different countries with the respective conditions for production of wine grapes. However, many of these regions have a warm-tropical climate: Oliveira *et al*., (2006) reported the chemical characteristics of Syrah grapes grown in a Brazilian tropical semi-arid region (State of Pernambuco) during four growing seasons (two calendar years, 2016 and 2017).

As evidence of the influence of different environmental conditions on the composition of the wine, Zurga *et al*., (2019) conducted a study on the phenolic content of autochthonous Croatian varieties (Plavac Mali and Teran) compared to that of non-autochthonous varieties such as Merlot and Cabernet Sauvignon, grown in Croatian regions: according to the autochthonous wine had a higher total content of compounds phenolics and (+)-catechins, thereby in addition to the genetic factor of each variety, the differences in terroir conditions had a significant impact on the values of these parameters. In these terms, studies have also been conducted in many other regions, such as Spain, especially with regard to the phenolic composition of white wines produced by autochthonous Alvarinho, Godello (..) and non-autochthonous Chardonnay, Riesling (..): even in these analyses, differences in the phenolic composition were highlighted, which was increasingly greater in the case of native varieties. In Europe, the adaptability of international grape varieties outside their terroir of origin, creates wines with different chemical and sensory profiles.

Cosme *et al*., (2009) studied the tannic profile of several monovarietal wines obtained from different Portuguese autochthonous (Touriga Nacional, Trincadeira and Castelão) and non-autochthonous (Syrah and Cabernet Sauvignon) red grape varieties grown in the Lisbon wine region during two vintages (2004 and 2005), highlighting a different phenolic composition. All these studies wanted to demonstrate how the composition of grapes and wine of the same vine varieties, but grown in different wine regions of the world, depends on various environmental factors and above all on the terroir; however, most of the studies have been conducted on the comparison between native and non-native varieties, and on the differences that emerged from them.

1.1 Brazilian Climate and Viticulture

Three different wine-growing areas are currently being commercially explored in Brazil and wines with different qualities and typicity are produced. The first is found in the South and South-East regions, where "traditional wines" have been produced for a hundred years. The second is located in the North-East region, where tropical viticulture has been practiced since 1985 for the commercial production of "tropical wines". The third is the most recent area, where the so-called “winter wines” have been produced in the South Eastern, North Eastern and Central Western regions of Brazil since 2004. Brazil is the only country in the world where these three wine regions are possible and information about these areas will be detailed in this chapter. The two factors that allow these possibilities with great variability are due to the climatic conditions and the management of the vineyard.

Brazilian viticulture occupies an area of 80,000 hectares and, of the 353 million liters of wine and derivatives produced in 2016, for the vast majority, comes from grapes of the *Vitis labrusca* species and their hybrids. The production of wines from *Vitis vinifera* represents only 19.6 million liters per year, mainly from the state of Rio Grande do Sul and, to a lesser extent, from the states of Santa Catarina, Bahia, Pernambuco and the southeastern states of Minas Gerais, São Paulo and Rio de Janeiro. The predominant climate is temperate humid, and viticulture is practiced in all three states, in Paraná, Santa Catarina and Rio Grande do Sul (Wrege *et al*., 2012).

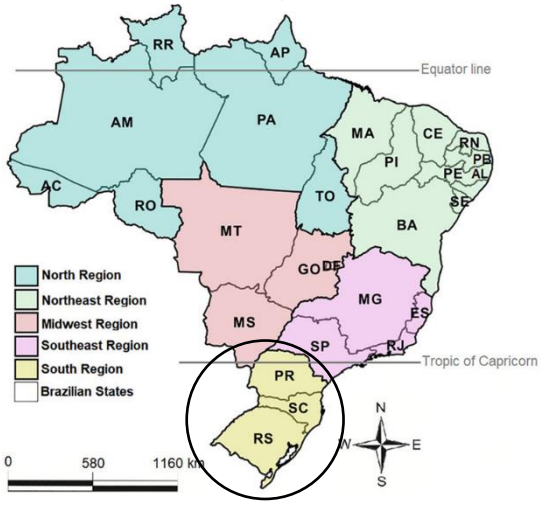


Figure 1. *Map of Brazil showing the states belonging to each region. Paraná—PR; Rio Grande do Sul—RS; Santa Catarina—SC; (Alvares et al., 2013)*

Serra Gaúcha (Gaucha mountain), the most famous and traditional wine region of Brazil, is located in the state of Rio Grande do Sul, consisting of 26 municipalities (Wrege *et al*., 2012). The average annual temperature of the region is about 17ºC. Brazil's second largest wine region is quite new and started in the mid-1980s. Precisely, the first commercial wines were sold in 1986 (Pereira *et al*., 2011).

The region is located in the northeast and the tropical wines are produced in the Vale do São Francisco (Valley of São Francisco), in a semi-arid tropical climate, where the

average annual temperature is 26.5ºC. The main feature of the region is that winegrowers can carry out two pruning and two harvests a year from the same vine (Tonietto and Pereira, 2011; Pereira *et al*., 2016). The third region of Brazil is the one that produces wines in winter: in this type of management, the harvest takes place in winter, and Brazil is the only country in the world where grapes are harvested in this season, because the temperatures can vary from 0°C (in the morning, sometimes with frost) to 25°C (afternoon).

Campanha Gaúcha is characterized by flat terrains, has a high number of hours of light, and dry summers, ensuring complete ripening of the grapes. The southeastern region of Rio Grande do Sul has pronounced ripples, located at altitudes between 400 and 600 m, experiencing dry, sunny summers with cold nights, and stony ground; Merlot and Cabernet Franc are the outstanding varieties. In the northern plateau of Rio Grande do Sul, at 1000 m a.s.l., the region of Campos de Cima da Serra has a characteristic high solar incidence, also due to low night temperatures. The altimetric region of the State of Santa Catarina has characteristics similar to those found in the Campos de Cima da Serra, with vineyards located between 900 and 1400 m; the slow maturation favors the conservation of acidity and high levels of aromatic compounds, which give freshness and typical white wines, respectively, in particular those obtained from Sauvignon Blanc.

Located at average altitudes of 900 to 1100 m, the region of Greater Curitiba with hot days and mild nights stands out for the production of medium-short cycle varieties, because it has humid summers which favor the onset of fungal diseases. The southern state of Minas Gerais has average altitudes of 800 and 1000 m, where the double pruning technique has been adopted, which causes the grapes to ripen during the winter, when there is a dry season with mild temperatures, making it an ideal place to produce high quality Syrah wines. In the State of São Paulo, there are altitudes between 1,000 and 1,300 m a.s.l. where the cool nights and the excellent sunshine during the day provide a thermal amplitude of 10°C at the time of the harvest. To this is added a dry, well-drained and granite soil, especially for the cultivation of Syrah and Viognier. The São Francisco River Valley features flat terrain, at an altitude of 400 meters. Located outside the area suitable for growing vines, this semi-arid region has low rainfall (less than 500 mm per year) and strong insolation. Its permeable clayey soils have proved to be suitable for the acclimatization of vines such as Moscatel, Cabernet Sauvignon and Syrah. However, despite the existence of several improvements that facilitate the development of the winemaking process, there is a localization movement in Brazilian winemaking: the growth of winemaking outside the traditional region (Serra Gaúcha). This localization

movement of viticulture has allowed the discovery of new terroirs in Brazil, allowing for the elaboration and marketing of differentiated products.

Therefore, it is a determining factor in the evolution of Brazilian winemaking. In this context, the aim of this work is to characterize the new Brazilian wine regions, describing their edaphoclimatic and productive characteristics, the types of wine and their importance in the evolution of Brazilian wine (Nicolli *et al*., 2015). Brazilian viticulture presents a great diversity. The culture is widespread from Rio Grande do Sul, at 31°S latitude, to Rio Grande do Norte and Cearra, at 05°S latitude. Elevation variation is also notable, with significant environmental diversity between production areas, including temperate, subtropical, and tropical regions. In recent years, the cultivation of vines on the West Frontier of the State of Rio Grande do Sul, has established itself among producers as an alternative of cultivation, driven by the ease of mechanization, due to its flat relief and above all due to the edaphoclimatic conditions which allow the production of grapes and the elaboration of fine wines with unique characteristics. The long days, with a large period of light for the plants, and the large temperature difference between day and night, favor the cultivation of the vine. The favorable conditions are complemented by the soil, rich in granite and limestone.

1.1.1 Rio Grande do Sul

Viticulture really took root in Rio Grande do Sul after the formation of the colonies of Italian immigrants, in a colonization process that began in 1875. The Italian colonizers brought the techniques of vine cultivation and wine production. In the 1960s, external capital investments were made, and multinational enterprises were located in the northeaster and southern regions of Rio Grande do Sul. These processes have made it possible to implement new cultivation systems that have led to changes in the circuit of the wine production area, especially in the relationship between the grape producer and the cellar.

Numerous studies have been conducted in the Serra do Sudeste region, in the state of Rio Grande do Sul. In the 1970s, scientific research by the Agricultural Research Institute of the Agriculture Secretariat of Rio Grande do Sul (IPAGRO), highlighted the edaphoclimatic potential of the Serra do Sudeste for vine cultivation. Currently, the total production of these municipalities is 787 hectares of vineyards and about 3,342 tons of grapes, which corresponds to more than 50% of the value of the entire agricultural production of the Serra do Sudeste. The state of Rio Grande do Sul from the

physiographic point of view, is formed by a relief of "gentle curves". The subsoil is made up of rocks of various ages, with associations of metamorphic, igneous and sedimentary rocks. The altitudes vary from 75 to 500 meters. The landscape features a large amount of boulders mixed with field vegetation with areas of gallery forest. The climate is characterized by regular rainfall, with an average of 1,367 mm and 1,444 mm per year for the region, interspersed with periods of drought. The mean annual temperature is between 17.6°C and 20.2°C (Protas *et al*., 2006).

1.1.2 Santa Catarina

A new wine region has been definitively established in the highlands of Santa Catarina. The paradigm shift with respect to the traditional viticulture practiced for many years in the State of Santa Catarina is due to the use of parameters that constitute the purest notion of terroir, i.e. the particularities involving the local climate, soils, grape varieties and human factors responsible for the development of this vineyard. In southern Brazil, at the highest latitudes and in places close to 1,000 meters above sea level, particular climatic conditions slow down the vegetative cycle of the vine, favouring the obtaining of the raw material for making the wines and allowing the ripening of the grapes which gives them an intense colour, specific aromas, good volume and gustatory balance. Studies have also been conducted in Santa Catarina to test the adaptability of the vines to this region; it all started in the 1990s with the installation of collections of Vitis vinifera cultivars to test their adaptability to the great climatic diversity of Santa Catarina. These tests were conducted, among other things, in the extreme conditions of São Joaquim, one of the main areas of Santa Catarina, at an altitude of 1,400 m, where the frequent spring frosts led to the elimination of most of the varieties, although some, such as Cabernet Sauvignon, have survived. The small experimental production turned out to be interesting.

The state of Santa Catarina is climatically characterized as C-mesothermal, with cool summers and a subtropical character, the climate of the vineyards of Santa Catarina has the general characteristics of temperate climates. The altitude, between 900 and 1,400 m a.s.l., at a latitude close to 28° south, influences the vegetative cycle of the vine, delaying the start of budding until October and, in some cases, prolonging the final ripening until the end of April. Grapes produced in regions of altitude above 900 m have their own characteristics and are distinct from those cultivated in other areas of the country, as well as phenolic maturation adequate to the elaboration of wines. The climatic

potential of the high-altitude regions to produce vinifera grapes (*Vitis vinifera L.*) in Santa Catarina, has been proven through several surveys. These conditions provide a differentiated climate in relation to the other wine regions of Brazil, interfering mainly in the phenological cycle. Due to the milder air temperatures in these regions, the vegetative and reproductive cycle of the grapevine is more extensive, resulting in slower ripening of the grapes to produce fine quality wines. In addition, it causes maturation in less frequent months (April and May) and amount of rainfall, providing grapes with greater sanity and oenological quality. In mountain climates, the hours of sunshine are beneficial for the development of maturation, easily allowing to obtain high levels of alcohol, determining a greater incidence of ultraviolet rays which act on the immune system of plants, favouring the formation of polyphenols and anthocyanins.

These polyphenols are important for enhancing the colour of wines, especially resveratrol, which is present at levels more than double those normally found in wines from other regions of Brazil. Low night temperatures cause a hormonal change which leads to a reduction in vegetative growth and the beginning of ripening with an accumulation of sugars, phenols and aroma precursors. Because nights are cold during maturation, the breakdown of malic acid is reduced, resulting in levels in musts and wines that are up to three times higher than normally encountered. The high concentrations of this acid give white wines a delicate acidity and sparkling whites and rosés the typical and exclusive liveliness of malic acid. In red wines, it makes malolactic fermentation essential and difficult to carry out, since the low ambient temperatures, the quantity of alcohol and the high polyphenolic load require cellars to be prepared for this task. In Santa Catarina, the soils are mainly of basaltic origin, structured brown earth and the predominant texture is sandy clay but differs in depth and quantity of stones. The Santa Catarina vineyard has recently stood out in front of other producers of fine wines in Brazil (Rosier, 2020).

1.1.3 Paraná

Paraná is the fourth largest grape-producing state in Brazil, and the cultivation of *Vitis vinifera* L. cultivars for winemaking is expanding to several regions of the state. The viticultural climate of some regions of the state of Paraná belongs to the climatic groups in which several traditional wine regions are located in the world, showing the potential for the expansion of winemaking in the state. The viticultural climate, associated with the latitude of Paraná and the thermal conditions for the year-round cultivation cycle of the

vine, make it possible to shift production to the western, northern and north-eastern regions and produce the best quality grapes for vinification in the autumn and winter period, due to the more favorable cold night index of rainfall and the lower volume.

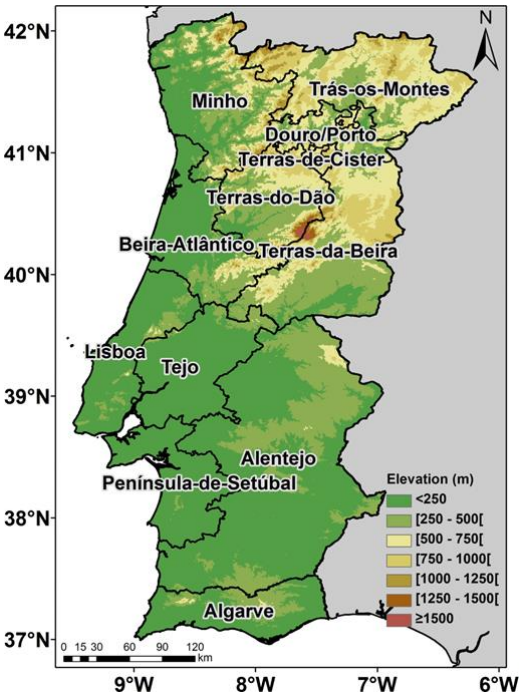
In the coldest regions of the state (Centre, South and East) it is possible to have only one production cycle, since the risk of frost prevents the exploration of the grapes in different periods, showing the potential for the expansion of winemaking in the state. Northern and north-eastern regions and the production of the best quality grapes for winemaking in the autumn and winter period, thanks to the more favourable cold night index and the lower volume of rainfall (https://www.embrapa.br/busca-de-publicacoes/-/publicacao/1091335/agroclimatic-zoning-for-winemaking-grape-production-in- the-state-of-parana).

1.2 Portugal Climate and Viticulture

In Portugal, the climate is pleasantly mild, being influenced by the Atlantic Ocean. The climate is cool and rainy in the north, gradually becoming warmer and sunnier when moving southward; in the far south, the Algarve has a dry and sunny microclimate. In the island areas, on the Spanish border, the climate becomes slightly continental. In the center-north, there are also mountain ranges. The summer is sunny everywhere, because in this season Portugal is protected by the Azores anticyclone; however, the tail end of an Atlantic perturbation may pass through the north from time to time. During the rest of the year there is no lack of rain, which is more frequent and abundant the further north one goes. For this reason, the landscape is very green in the north and gradually becomes drier going southwards, until we reach the Algarve, which has a fairly dry climate. In fact, the annual rainfall, which amounts to 1,450 mm in Braga and 1,100 mm in Porto, rises to around 900 mm in Coimbra, 800 mm in Lisbon and falls to around 500 mm in the Algarve.

The wettest season is winter. Winter, from December to February, is mild on the coasts, including the northern ones, as the average temperature in January goes from 10 °C in Porto, to 12 °C in Lisbon, to 12.5 °C in Faro. In winter, there are periods of good weather, as the Azores anticyclone is able to reach the country even in this season, but there are also waves of bad weather, with rain and wind. At times, the wind can blow with gale force, especially in the north. The location on the ocean provides good shelter from cold currents and night frosts, which are actually very rare and, in any case, not intense,

especially on the coast, where records are a few degrees below zero in the north and around 0 °C in the south.



Instead, in the island areas of the center-north, the cold is more intense, especially in the hilly and mountainous areas, where it can sometimes even snow. Summer, from June to mid-September, is sunny everywhere, and is mild or even cool on the northern coasts, and hot in the center south. In Porto, the August average is 20 degrees, and daytime highs are around 25 degrees. In Lisbon, the August average is higher, reaching 23.5 °C, with highs of 28 degrees. However, in the coastal areas most exposed to the ocean winds (see Peniche, Sines, Sagres), it is cool even in summer (Costa *et al*., 2019).

***Figure 2**. Grapevine growing regions in mainland Portugal as defined by the Instituto do Vinho e da Vinha (IVV)*

1.2.1 Dão Region

Dão is one of the most important Portuguese wine region with the Dão-Lafões sub-region of Centro, in Portugal; it is one of the oldest wine regions in Portugal as well. Dão wine is produced in a mountainous region with a temperate climate, in the area of the Rio Mondego and Dão rivers, in the central-northern region of Portugal. The region became a Denominação de Origem Controlada (DOC) appellation in 1990. The Dão region is the origin of the Touriga Nacional grape variety, which is the main component of Port wine. The 80% of the region's production is made up of red wines, and the DOC regulation stipulates that at least 20% of the production is made up of Touriga Nacional. The location of the Dão region keeps its temperate climate sheltered from the cold and rigors of the Atlantic Ocean. The region receives abundant rainfall in the winter, which keeps the soils hydrated during the hot, dry summers. Cooler nights and warmer days allow for a slower maturation process, producing a strong aroma and acidity that result in rich and elegant wines (Costa *et al*., 2019).

1.2.2 Vinhos Verdes Region

The demarcated region of Vinho Verde extends in the north-west of Portugal, in the area traditionally known as Entre-Douro-e-Minho. The northern border is formed by the Minho River, which is part of the Spanish border, the southern one by the Douro River and the Freita, Arada and Montemuro mountains, the eastern one by the Peneda, Gerês, Cabreira and Marão mountains and the western one by the Atlantic Ocean. In terms of geographical area, it is the largest Portuguese demarcated region and one of the largest in Europe. The Atlantic influence, the predominantly granite soils, the mild climate and the high rainfall, are reflected in the freshness, lightness and elegance of the wines of this region. Variations in soil types and microclimates justify dividing the region into nine sub-regions, with different grape varieties recommended for the production of still, liqueur and sparkling wines.

The main important sub- region known for the production of Alvarinho includes the districts of Monção and Melgaço. The sub-region of Monção and Melgaço has a very particular microclimate and is therefore cultivated exclusively with the Alvarinho (white) and Pedral (red) varieties and shares the recommendation for Alvarelhão (red) with the Baiao sub-region. All three varieties are early ripening. This microclimate is characterized by cold winters with intermediate rainfall, while summers are very hot and dry, indicating a limited Atlantic influence (Jones *et al*., 2012).

1.3 Touriga Nacional

One of the most common variety in Portugal is the Touriga Nacional, a *Vitis Vinifera* sub. sativa red variety. Some important features are the high vigor, low yields, and the small dark blue berries (particularly rich in phenols compounds). It cannot develop in insufficient sunlight or in region with high drought stress (Santos *et al*., 2017). It's well known that Touriga Nacional is cultivated in other states, as Brazil, South Africa, California (especially to do some fortified wines): the tropical climate in some zones (as São Francisco Valley) allows to do multiple harvests in one year, by the way some differences can be found in grapes composition between first harvest and second harvest. Studies carried out that grapes and wines obtained by first harvest have a better quality in sensory analysis, especially in acidity, body, floral aromas and limpidity: this sensory characterization also occurred in analytical parameters. Sugar, tonality and pH

were higher in second harvest, while total acidity, polymeric tannins, polymerization index were higher in first harvest (Oliveira *et al*., 2018).

***Figure 3**. Touriga Nacional grapes collected from the Brazilian vineyards on which the study is based*

In region with altitude > 300 meters as Douro region, the yield is lower because of lower cluster weights, and lower berry volumes. This effect might be the result of reduced soil fertility and water availability at higher altitudes. As well, exposure plays an important role in yield: best results were achieved with a S-E and N-E exposition (Oliveira and Magalhães, 2004). Touriga Nacional is used mainly in Douro, but also in the other Portugal wine regions, where is blended with up to 80 varieties as Touriga Franca, Aragonez etc. Dão winemaking region has a high viticulture potential but, due to the high variability climate in years, Touriga Nacional's health and composition (total acidity, total soluble solid, anthocyanins content etc.) may be compromised (Pedroso *et al*., 2012). Douro winemaking region is a particular Portugal region famous all over the world thanks to wines quality. The 70% of the vineyards are planted with a steep slope, higher than 30% (this is the reason why Douro Valley is part of the "World heritage list UNESCO")(Queiroz *et al*., 2013).

This variety is used to create common table dry wine and in the blends of Porto wines, but it is not famous only in Portugal: it has been spread worldwide. The high tannins concentration gives structure and body to the wine, allowing a long aging to wine, especially in wood (Santos *et al*., 2017). Touriga Nacional is characterized by small, thick-skinned grapes, with a high skin/ volume ratio and high anthocyanin pigment content that results in a higher release of anthocyanins into the must during the maceration process (Jordão *et al*., 2012); as is well known, anthocyanins are released especially during first fermentation phase. The black fruits flavours and fruity-citric aroma (due to the presence of linalool, linalyl acetate (Silva Ferreira *et al*., 2006) and C13-Norisoprenoids give a particular bouquet to the wine. Oliveira *et al*. (2006) showed that Touriga Nacional has a low-medium concentration in 12 carotenoids (927 µg/kg of

grapes as average of total carotenoids of 2001-2002-2003 vintages), a low concentration in bounded C13-Norisoprenoids and high concentration in free C13- Norisoprenoids. In contrast, a good concentration of b-ionone (3.1 µg/L as average of 2001- 2002-2003), 1,1,6-trimethyl-1,2-dihydronapthalene (TDN) (10 µg/L as average of 2001-2002- 2003), and vitispirane (9 µg/L as average of 2001-2002-2003) were founded in the wine. These results can explain, in part, the floral and violet aroma characteristics of Touriga Nacional wines.

A trial regarding Touriga Nacional's aroma descriptor (Silva Ferreira *et al*., 2006) showed that the cultivar is particularly rich in a-pinene (described as pine-like aroma), g-terpinene, (E)- b-ocimene and b-phellandrene (which were important for the bergamot essential oil aroma). Petronilho *et al*., (2020) described Touriga Nacional's aroma also like tree, tropical, and berry fruit; in the same study, the cultivar expressed also a sweet and oxidized note, with a toasted aroma, probably due to 2-methyl-3-furanthiol. The study also showed a good composition in lactones (g-nonalactone, g- and d-decalactone), phenols (guaiacol, eugenol, 3-propylguaiacol, and 4-vinylguaiacol) but in a small quantity. Falqué *et al*., (2004) formed a panel test and analysed Touriga Nacional wines aroma describing it with plum brandy, dry raisin, wild fruit aroma-like.

About pathological status of TN, it is proved that the variety is mildly/severely susceptible to *Plasmopara* viticola infection (downy mildew) with an important sporulation rate, necrosis rate and oil stains (yellow area visible on the upper leaf surface)(Boso *et al*., 2008). The variety is highly susceptible to *Botrytis* cinerea attack (in France, Grande Ferrade site): more than 98% incidence was diagnosed, together with cultivar as Aragonez and Cabernet Franc (Paňitrur *et al*., 2018).

Regarding the Touriga Nacional grown in Brazil, other studies were carried out with the aim of evaluating the polyphenolic composition, other oenological parameters and the sensory profile; just in the São Francisco Valley it is possible to carry out two harvests a year, one in the first and one in the second season of the year with higher temperatures that favour rapid ripening of the grapes. The results showed that there were differences in the polyphenolic composition due to the different harvest times and also at a sensory level, the floral aromas rated with the highest scores were found above all in the first part of the year when temperatures are not excessive and drought is moderate, compared to the second semester (Tonietto *et al*., 2012). Colorimetric parameters such as total anthocyanins have been evaluated (Ribéreau-Gayon and Stonestreet, 1965); coloured anthocyanins (Somers and Evans, 1977); total phenols (Ribéreau-Gayon, 1970); phenols, flavonoids and non-flavonoids (Kramling and Singleton, 1969); colour due to copygmentation (Boulton, 2001); colour intensity and tone; total and polymeric pigments

(Somers, 1971; Somers and Evans, 1977) and tanning power (De Freitas and Mateus, 2001). These parameters were determined according to methods proposed and described in the literature.

The grapes harvested in the first half of the year had a higher quantity of tartaric acid and this also depends on the specific variety, Touriga has a total acidity with values between 4.5 and 6.0 g/L; the fact that the grapes harvested in the second half of the year had lower total acidity may instead be due to the fact that there was a greater degradation due to high temperatures: this is especially true for malic acid which is degraded as quickly as the higher the temperatures. The concentrations of phenolic compounds such as total flavonoids and non- flavonoids also remained higher in the first half year than in the second half, in line with acids. The concentration of anthocyanins is mainly influenced by temperatures and the ideal range of accumulation would be between 15 and 17 degrees at night and 24-26 during the day, so the concentrations of anthocyanins were lower in the second half of the year having temperatures higher than 35 which favored the degradation of anthocyanins during maturation; however a higher concentration of anthocyanins may also be due to the characteristic of the variety which favors a lower degradation of anthocyanins. Furthermore, a higher presence of anthocyanins esterified with acetic acid was found in Touriga Nacional grown in Brazil, compared to that grown in the Douro region of Portugal. In terms of tannins, the effect was mainly due to the vintage rather than the season; moreover, according to what was found by Cosme *et al*., (2009) for the same cultivar, fewer tannins were found in Brazilian Touriga than in those grown in Portugal.

Non-flavonoid compounds were significantly affected by harvest season rather than by vintage, and significant differences in total phenols and flavonoids were found, with higher concentrations in Touriga of Brazil than in those grown in Portugal. Furthermore, the concentrations of total and colored anthocyanins were also lower in the Brazilian varieties compared to the Touriga of the Dão region: the higher temperatures in Brazil led to a greater degradation of anthocyanins, so the total concentration is lower overall according to a warmer climate. Furthermore, a lower monomeric anthocyanin content was found in Brazilian Touriga than in Portuguese Touriga, for the same reason, according to the analyses reported by Mateus *et al*., (2002) The results for the monomeric, oligomeric, and polymeric fractions of condensed tannins in Touriga Nacional show an effect of the harvest on the composition of condensed tannins in Touriga Nacional wines. Monomeric and oligomeric tannins presented higher values in wines from the second harvesting season, while polymeric tannins reached higher values

in the first harvesting season (Oliveira *et al*., 2018). According to what emerges from the analyses, it would therefore seem that the concentration of flavanols is influenced by the harvest period but above all by the climatic trend of the different regions and states, such as Portugal and Brazil, and possibly by the different microvinification technologies, which can transfer with crushing, maceration or fermentation, a greater or lesser amount of procyanidins (Cosme *et al*., 2009).

1.4 Alvarinho

Alvarinho, it is a variety for which it is not clear whether it comes from Galicia or from the north of Minho (Monção). To date, the region of greatest expansion is the sub-region of Monção. It can also be classified with official synonyms (national and OIV) such as Albariño and with historical and regional synonyms, such as Galego, Galeguinho (Ponte de Lima); Truel (1986) cites Cainho Branco.

***Figure 4**. Alvarinho grapes collected from Brazilian vineyards on which the study is based*

The current vineyard area in Portugal dedicated to its cultivation is equal to 1.800 ha, while in Spain it is 5.49 ha, with a national use of 1.35% in Portugal and growing in Spain (https://www.vinha.pt/wikivinha/section/casta-vinho/alvarinho/). The material used is polyclonal from RNSV, and the certified clones are 44-47 ISA, 42, 43 JBP. In Spain there are more than a dozen clones certified in Galicia, including A-062 and A-123. In terms of regional classification, the «Vinho Verde Alvarinho», «Vinho Verde Alvarinho Espumante» is considered a DOC quality wine in Portugal, specifically in the Monção sub-region. In terms of resistance and sensitivity, it is a variety that is sensitive to high water stress, shows good resistance to sunlight and wind; it is sensitive to powdery mildew, inclined to Esca and less sensitive to Botrytis, while it is sensitive to mites. Alvarinho is mainly cultivated in soils derived from granite, favourable for obtaining a quality wine, whereas it is less suited to heavy, humid and poorly drained soils. It is a vine that develops well in a climate with high insolation and moderate presence of water and does not present any problems with the rootstocks generally used in the region of development.

Alvarinho has good oenological potential and can be a high-quality table wine, sparkling wine and brandy. The alcohol content of the must is medium-high, equal to 12% vol, and has a high natural acidity (5.5-7 mg/L); the total polyphenol index of the must is between 325 and 948 mg/L, depending on the oenological technology used. Alvarinho is a variety moderately sensitive to oxidation, it has an intense colour and a straw yellow tone, with citrus reflections. In terms of tannins, it has monomeric values generally between 4.0-27.0 mg/L, oligomeric between 11.0-180.0 mg/L and polymeric between 47.0 and 735.0 mg/L (values obtained with pellicular maceration). Following laboratory analyses conducted on the aromatic components of the wine, it emerged that this variety has a mainly floral, tree-like, and fruity character, especially tending towards tropical fruit, with excellent structure, persistence and balance (https://plansel.com/viveiros/viticultura/airen/)(Bohm, 2010.)

As in the case of Touriga Nacional, a study was also conducted for Alvarinho to compare various oenological parameters and the phenolic characteristics of this variety with others, depending on the different genotype and cultivation environment. Edaphoclimatic conditions can include temperature, rainfall, soil water content, composition and structure, evapotranspiration, and relative humidity; these can clearly influence the chemical profile of the grapes of the Alvarinho, cultivars grown in Dois Portos (southern Portugal) and in northern Portugal. It should be emphasized that the microclimate and cultural practices of the vineyard can greatly influence the physiology and growth of the vine and the composition of the berries (Santos *et al*., 2013). Data relating to the sugar concentration of the berries was collected, depending on the stage of development, the environment and the different agronomic practices: in this context, a number of metabolomic analyses have been carried out to compare grape berry composition at various developmental stages (Ali *et al*., 2011), or to look at differences between cultivars and growing seasons (Pereira *et al*., 2006) or regions (Son *et al*., 2009). The study aimed to characterize Alvarinho in terms of chemical composition and different sugars were detected: glucose, fructose and, in smaller quantities, sucrose which represent the most important sugars in all varieties and regions, and the quantity of sugar is increased from veraison to the ripening stage. Significant differences in ripe sugar content between cultivars and regions were not expected, because maturity was considered when soluble solids reached 18º Brix for all varieties and regions. However, there was a very consistent difference between North and South regarding sugar composition: the South region, being hotter, stimulated the invertase activity (Li *et al*., 2012).

Other parameters were also analysed, and the results also showed relatively high levels of malic acid and low levels of tartaric acid in ripe Alvarinho grapes grown in the North

compared to two other varieties. Furthermore, ripe Alvarinho grapes contained much less tartaric and malic acid in the South than in the North. Therefore, the sampling location actually influenced the levels of tartaric and malic acid in the grapes. In the highest temperature area, the concentration of malic acid decreases, while vines grown in colder climates show higher quantities of malic acids (Pereira *et al*., 2006). Studies have also focused on the content of phenolic compounds: it has been reported that flavan-3-ol compounds account for 36% of total non-anthocyanin polyphenols and that procyanidin B1 is the most abundant flavanol in grapes, which it represents 64% of the total flavanols. Catechins represent on average 20% of total flavanols, epicatechin and epicatechin gallate represent no more than 10% of total flavanols (Chen *et al*., 2006). The study identified only (+) catechin and its isomer (-) epicatechin: both catechins and epicatechins decreased significantly from North to South suggesting that their levels in ripe grapes depend on the growing region. When ripe, the highest concentration value was found in Alvarinho grapes grown in the North, probably due to the contribution of anthocyanins. In Alvarinho total phenolics were much higher in the northern part of Portugal than in the southern part, because of the light and temperature, higher in the south; sun exposure of grape bunches can affect the flavanol content of grapes. In a previous study it was demonstrated that the total content of phenols in grapes from shaded clusters was lower than in grapes from exposed or moderately sun exposed clusters (Price *et al*., 1995).

These studies conducted on Touriga Nacional and Alvarinho are aimed at proving how the chemical composition of the two varieties can vary considerably also according to the soil and climatic conditions of one country of cultivation compared to another, both in terms of area (North or South) of cultivation within the same country.

1.5 Objectives

Due to the lack of sufficient technical and scientific studies about the differences of these two varieties in different countries, the aim of this work was to analyse the phenolic composition, the colour and the sensory profile wines produced from two Portuguese varieties, Alvarinho and Touriga Nacional cultivated in different areas of Southern Brazil: Rio Grande do Sul, Santa Catarina and Paranà.

In this regard, the following scientific work wanted to prove how the same varieties, but the native and non-native ones, could be different.

To respond to this particular challenge, several essays were made in a semi-industrial scale, with a collaborative research work, using Touriga Nacional and Alvarinho wines provided by Polytechnic Institute of Viseu, where the vinification took place.

2. Material and methods

2.1 Vineyard locations and vinifications

The study is based on comparing the data obtained as a result of analyses carried out on various parameters. The analyses are conducted with the aim of comparing the oenological characteristics of wines obtained from two varieties of Portuguese origin with those of wines obtained from the same varieties grown in Brazil previously mentioned, using the Portuguese variety samples as control samples for reference.

Touriga Nacional grapes were harvested in 2022 at the technological stage of ripeness from vineyards ESA/IPViseu, located in Viseu, in Portugal and from Vinícola Quinta de Neve, São Joaquim, in Santa Catarina State, and Vinícola Família Lemos de Almeida, Capões, in Rio Grande do Sul State sites, in Brazil.

The winemaking process followed the standard red wine technology production with a maceration time of 6 days at 25 ± 2°C. After destemming and crushing, the sulfitation of the grape must by the use of potassium metabisulfite (0.07 g/L) was followed by alcoholic fermentation using a commercial *Saccharomyces cerevisiae* yeast strain (Fermol Rouge; AEB Group, Brescia, Italy) and inoculated at 20 g/hL. During alcoholic fermentation, a yeast nutrient was added in the amount of 25 g/hL after the one-third of sugar has been fermented. After the end of alcoholic fermentation, the pomace was pressed. Wine was racked from lees 15 days after the end of malolactic fermentation, and then sulfited (readjusted to 30 mg/L for free SO_2). The red wine produced was kept in the stainless-steel tank under controlled conditions (20 ± 0.5 °C) and analysed for the free SO_2 level regularly until used in this experiment.

Alvarinho grapes were harvested during the vintage 2022 from the vineyards of the Casa da Gandarinha, Barcelos, in Portugal, from Vinícola Legado Campo Largo, in Paraná State and Vinícola Família Lemos de Almeida, Capões, in Rio Grande do Sul State sites, in Brazil.

The grapes were vinified following the classical procedure for white wine production. However, after the sulphitation of the grapes (50 mg/kg of $K_2S_2O_5$), crush and destemming, followed by a natural clarification process for 24 hours at 12 ºC. Then, the must was fermented in a stainless-steel tank using a standard *Saccharomyces cerevisiae* yeast strain (Fermol Arome Plus; AEB Group, Brescia, Italy) and inoculated

at 20 g/hL. During alcoholic fermentation, a yeast nutrient was added in the amount of 25 g/hL after the one-third of sugar has been fermented. The alcoholic fermentation process was completed in two weeks keeping the temperature below 19 ºC. After the alcoholic fermentation the wine was racked and removed from contact with the lees. After the fermentation the wine was kept in the stainless-steel tank under controlled conditions (temperature ± 18 °C), analysed for the free SO_2 level regularly (values periodically readjusted to 35 mg/L) until used in this experiment.

2.2 Experimental design

The general chemical-physical characteristics of the samples used in the study are reported in the following tables (Table 1. /Table 2.).

***Table 1**. General physiochemical characteristics of Touriga Nacional wines used in the present study*

	Touriga Nacional		
	Portugal PT	Rio Grande do Sul RG	Santa Catarina SC
Alcohol (V/V)	12,6	12,5	12,5
Total Acidity (g/L tartaric acid)	4,40	5,70	5,05
pH	3,30	3,30	3,30
Malic acid (g/L)	-	0,6	0,3
Gluscose+Fructose (g/L)	2,0	1,5	1,7
Volatile Acidity (g/L)	0,28	0,24	0,26
SO2 Free (mg/L)	35	35	35
SO2 Total (mg/L)	75	90	85

The analyses are carried out starting from 18 samples: 9 samples of Touriga Nacional (Table 1.), and 9 samples of Alvarinho (Table 2.). The samples were divided into 3 groups, each representing respectively a Touriga Nacional obtained from varieties grown in Portugal (TNPT), one from varieties grown in Rio Grande do Sul (TNRG) and one from varieties found in Santa Catarina (TNSC). Each group is in turn made up of 3 samples, each representing 3 different microvinifications (15L obtained for each microvinification) from which this wine was obtained (Figure 5.).

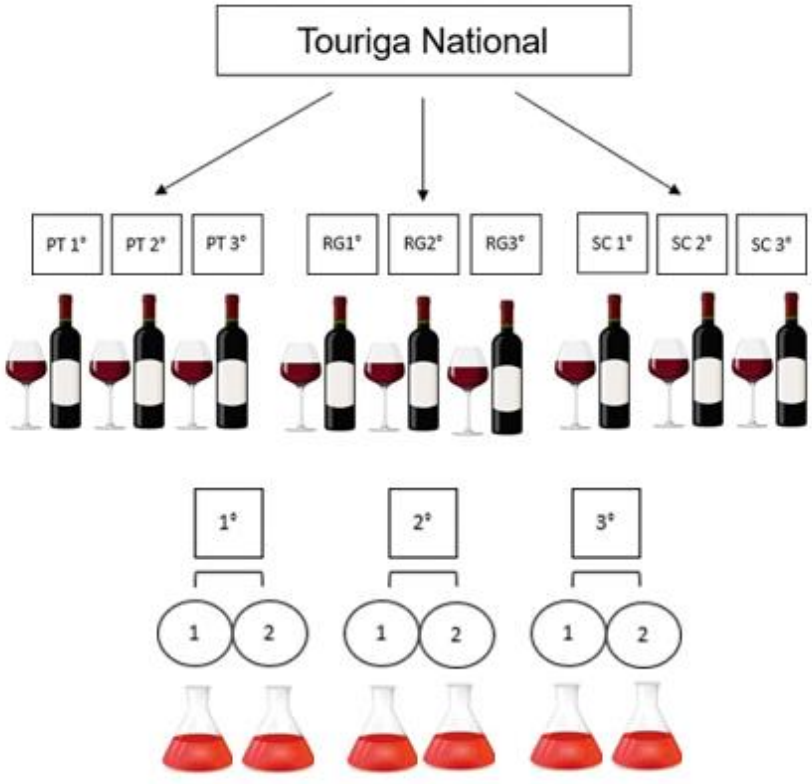


Figure 5. *Touriga Nacional experimental design*

Legend: 3 different microvinifications ***TNPT 1st, TNPT 2nd, TNPT 3rd/ TNRG 1st, TNRG 2nd, TNRG 3rd/ TNSC 1st, TNSC 2nd, TNSC3rd***

Each microvinification is evaluated twice (Sample ***1****/ Sample* ***2****)*

Also, in the case of the whites, the samples were divided into 3 groups, each representing an Alvarinho of Portuguese origin (ALPT), one obtained from varieties grown in Rio Grande do Sul (ALRG), and one obtained from grapes from the Paranà State (ALPR); each group is composed of three samples of Alvarinho obtained from 3 different microvinifications. Each microvinification is evaluated twice (Sample 1/ Sample 2), thus obtaining two samples, sample 1 and sample 2, and consequently two results for each microvinification in the case of the evaluated parameter (Figure 6.); the aim is to make an analysis as accurate as possible in statistical terms, reducing the error among the wine samples considered.

***Table 2**. General physiochemical characteristics of Alvarinho wines used in the present study*

	Alvarinho		
	Portugal PT	Rio Grande do Sul RG	Paraná PR
Alcohol (%)	14,5	9,9	11,2
Total Acidity (g/L tartaric acid)	6,65	5,50	6,00
pH	3,20	3,35	3,58
Malic acid (g/L)	2,0	1,3	2,6
Gluscose+Fructose (g/L)	1,0	2,1	1,8
Volatile Acidity (g/L)	0,50	0,27	0,32
SO2 Free (mg/L)	30	30	30
SO2 Total (mg/L)	90	90	85

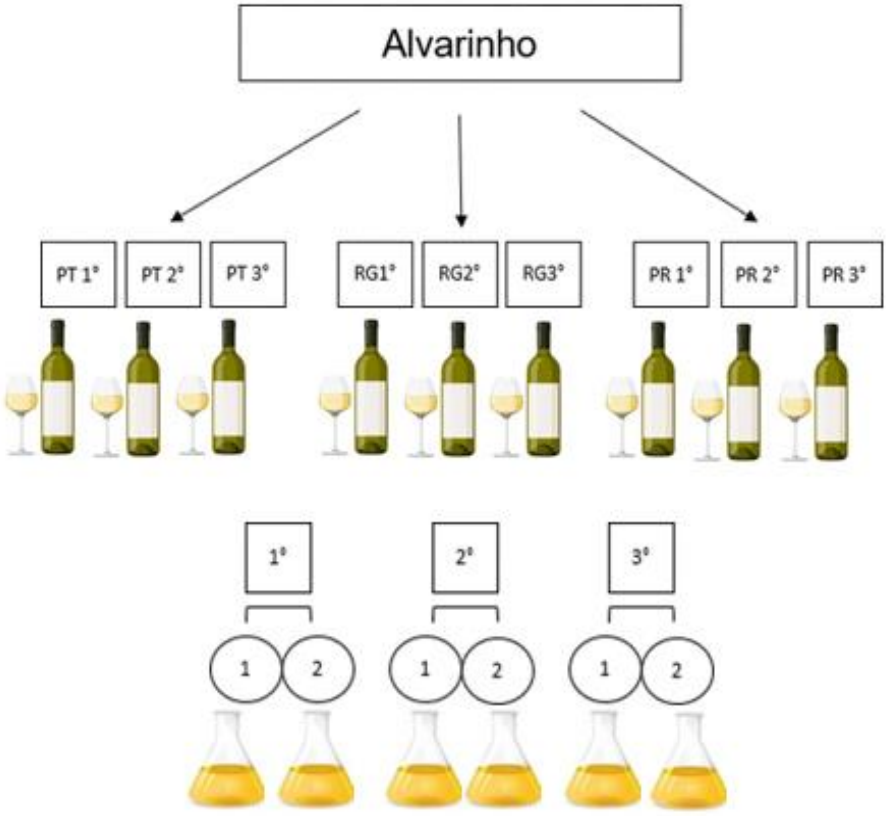


***Figure 6**. Alvarinho experimental design*

Legend: 3 different microvinifications ***ALPT 1st, ALPT 2nd, ALPT 3rd/ ALRG 1st, ALRG 2nd, ALRG 3rd/ ALPR 1st, ALPR 2nd, ALPR3rd***

*Each microvinification is evaluated twice (Sample **1**/ Sample **2**)*

Since in some cases variable results were found, in the case of the Touriga Nacional red wines grown in Brazil, the analyses were carried out on the first and third microvinification of TNRG and on the second and third microvinification of TNSC. The samples analysed are the result of microvinifications within which some reactions may have occurred that destabilized some parameters, reason why some of the microvinifications foreseen at the beginning were excluded during the study.

2.3 Analytical methods

The wine analyses were studied with different methods, which will be described in this chapter; moreover, the analyses are supported by the use of laboratory machines such as the pH meter, the turbidity meter, the spectrophotometer, the evaporator, the ultrasonic mixer. The analyses are conducted daily from the opening of the wines until the end of the scheduled analyses, in order to have the most correct and reliable data. The parameters analysed are tannin power, total phenols, flavonoids, non-flavonoids, colour with chroma metrics, such as a*, b*, L*, C* and h*, (each corresponding to red-green, yellow-blue, clarity, chroma, and hue angle), copygmentation, colour intensity, tonality, total anthocyanins, degree of ionization, polymerization index, ionized anthocyanins, total pigments, polymerized pigments, and amount of catechins and procyanidins, based on their polymerization grade and fractions. The wines are also subjected to a sensory analysis, with the aim of highlighting the sensory differences, in terms of consistency, colour, brilliance, olfactory and gustatory hints, aromas, balance and directness of the wine.

2.3.1 General phenolic composition

Kramling and Singleton (1969) proposed the following method: it is based upon the determination of the phenol content before and after precipitation and removal of the flavonoids through reaction with formaldehyde under selected conditions (low pH, room temperature, etc.). Under the selected conditions phenols lacking a *meta*-dihydroxy grouping (nonflavonoids in plants) did not precipitate.

Total Phenols: in volumetric flask (100 mL), it is necessary to put 1 mL of wine in each and then brought to volume with distilled water; to measure the absorbance for each sample at 280 nm with 10 mm square cuvettes, range 190 nm-1400 nm.

Non-Flavonoids (N-F): in a centrifuge tube, it is necessary to add 10 mL of sample with 10 mL of HCL (previously diluted 1:4), 5 mL of Formaldehyde (previously diluted at 36%: 2,08 mL in a flask of 100 mL brought to volume with water) and nitrogen. After it is necessary to store samples in a dark place for 72 hours (red wines). After a centrifugation for 10 minutes at 3500 rpm at 20 degrees, putting in a volumetric flask, 5 mL of samples are diluted with 45 mL of distilled water: the mix can be put in the spectrophotometer in order to read the absorbance at 280 nm.

Flavonoids: are determined by the difference between total phenols and non-flavonoids.

$$IPT\ (Total\ Phenols\ Index) = Abs280nm * 100\frac{mg}{L} of\ acid\ gallic$$

To calculate the amount of total phenols (ITP) quantifiable from the absorbance, in mg of gallic acid per litre, a calibration curve was created, in order to correlate the absorbance value at 280 nm with the total phenol value expressed in mg of gallic acid per litre, obtaining the following:

$$TP = A280 + 0.03440.038 \times 100\ (mg/\text{l of}\ gallic\ acid) \qquad R2 = 0.9962$$

$$\textit{Non-Flavonoids (mg/L-1 of acid gallic)} = \frac{[(Abs*10)+0.0344]}{0.038}$$

Flavonoids (mg/l-1 of acid gallic) = Total Phenols – Non-Flavonoid

2.3.2 Chromatic characterization

2.3.2.1 Colour intensity and hue

The "chromatic characteristics" of a wine are its luminosity and chromaticity. Luminosity depends on transmittance and varies inversely with the intensity of colour of the wine. Chromaticity depends on dominant wavelength (distinguishing the shade) and purity (OIV Method MA-AS2-07B).

A spectrophotometric method whereby chromatic characteristics are expressed conventionally, as given below:

The intensity of colour is given by the sum of absorbencies (or optical densities) using a 1 cm optical path and radiations of wavelengths 420, 520 and 620 nm.

The hue is expressed as the ratio of absorbance at 420 nm to absorbance at 520 nm.

For the determination it is necessary:

spectrophotometer enabling measurements to be made between 300 and 700 nm;

glass cells (matched pairs) with optical path b equal to 0.1, 0.2, 0.5, 1 and 2 cm.

If the wine is cloudy, it is clarified by centrifugation; young or sparkling wines must have the bulk of their carbon dioxide removed by agitation under vacuum. The optical path b of the glass cell used must be chosen so that the measured absorbance A, falls between 0.3 and 0.7.

The spectrophotometric measurements are taken using distilled water as the reference liquid, in a cell of the same optical path b, in order to set the zero on the absorbance scale of the apparatus at the wavelengths of 420, 520 and 620 nm. It is necessary to use the appropriate optical path b, read off the absorbencies at each of these three wavelengths for the wine.

The calculations and results expression are made following these steps:

calculate the absorbencies for a 1 cm optical path for the three wavelengths by dividing the absorbencies found (A_{420}, A_{520} and A_{620}) by b, in cm;

the colour intensity is conventionally given by

$$Intensity : A420 + A520 + A620$$

the hue is conventionally given by

$$hue : \frac{A420}{A520}$$

2.3.2.2 Colorimetric coordinates

CIE/Lab method allow users to evaluate colour attributes, identify inconsistencies, and accurately express their findings to others in numerical terms, in order to evaluate colour in a graphic (Figure 7). The study was carried out with a spectrophotometer (Model Cary 100 UV- VIS): after the wine was centrifuged, it was placed in the spectrophotometer with different cuvettes depending by specific analysis. A cuvette of 1 mm was used to

read the absorbance from 380 to 780 nm, using distilled water as reference “ZERO”, in order to establish the base line. The equipment setting must be with a data interval of 5 nm, with illuminates settled D_{65} and observers of 10* degrees.

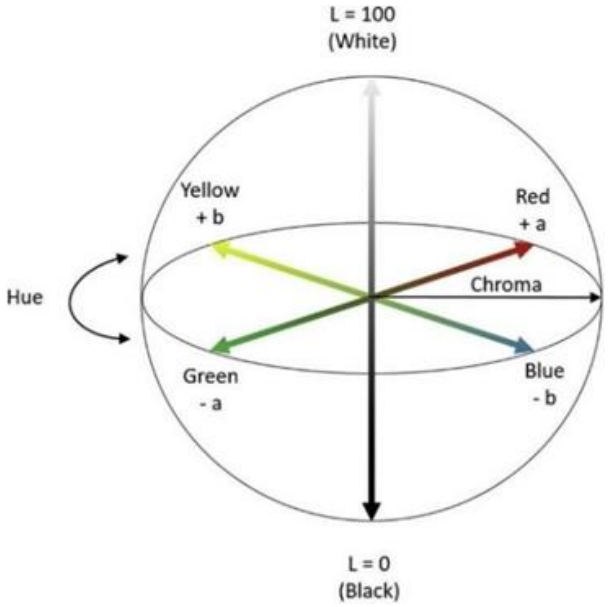


Figure 7. *CIELAB diagram colour space (Mcvoy 2015)*

The parameters found are: total colorimetric difference, like the clarity, related to luminosity, which is represented by L* value; a* and b* values, which represent the different colour components, the chroma C*, the angle of hue H*, expressed in degree and the H*C, the hue correction, which represents the difference between 360˚ of the total angle and the negative angle value found previously in H*. The schematic explanation of the parameters follows in table 3.

Table 3*. CIE/Lab parameters explanation (Method OIV-MAAS2-11(OIV 2006))*

Colorimetric coordinates	Symbol	Interval	Decimal
Clarity	L*	0-100 0 black 100 white	1
Red/Green colour component	a*	>0 red <0 green	2
Yellow/Blue colour component	b*	>0 yellow <0 blue	2
Chroma	C*		2
Tone	H*	0-360°	2

2.3.2.3 Copygmentation

Copygmentation is obtained from two absorbance readings, carried out following two different steps proposed by Boulton (2001).

Once the wine sample is centrifuged, acetaldehyde is added in wine in order to highlight the bonds between anthocyanins and SO_2 (10 mL of wine and 0,1 mL of acetaldehyde) and the first absorbance at "520a" nm is read; in the meanwhile, 1L of hydroalcoholic solution is made using 120 mL of Ethanol, 5g/L of tartaric acid, and distilled water to fill the volume, at pH 3.20, (all measurements are made on the basis of proportions calculated for the number of samples to be analyzed, in this specific case, 3 microvinifications of TNPT, the first and third microvinification of TNRG and the second and third microvinification of TNSC).

Wine is diluted with and hydroalcoholic solution in order to dissociate anthocyanins-copigment complexes. After 45 minutes 2,5 mL of wine is diluted in 50 mL of hydroalcoholic solution and the "520b" nm absorbance can be read.

In order to obtain the results, it is necessary to do the calculation:

$$CC\ (a.u) = 520a - 520b \qquad CC\ (mg/L\ of\ Malvidin\ 3\text{-}O\text{-}glucoside) = CC\ (a.u) * 20.3$$

2.2.3 Pigment evaluation and characterization

2.3.3.1 Anthocyanins and pigment contents

These parameters are evaluated on the basis of the anthocyanins and colour pigments present in the red wines analysed in the study. The evaluation method was developed by Somers and Evans, (1997); after centrifugation (5000 rpm for 10 minutes) it is necessary to use 1 mm cuvettes and then:

read absorbance at 420-520-620 nm, using distilled water as reference "ZERO";

add 5 mL of $K_2S_2O_5$ (20%) and then read absorbance at 520 nm;

add 100 mL of wine in 10 ml of HCL (1M) and then put the test tube in a thermal bath (25°C for 3-4 hours) and then read absorbance at 520 nm in a cuvette of 1 cm, using as references a solution with HCL (1M).

All the absorbance results have to be multiplied for 10 in order to correct the “10mm pathlength”; only the absorbance read after adding HCL, has to be corrected by multiplying for 101 dues to the dilution.

With the Somers and Evans methods is possible to evaluate these parameters:

Total anthocyanins (mg/L malvidin 3-O-glucoside) : $20 * (Abs520HCl - \frac{5}{3} * Abs520SO2)$

Degree of ionization of anthocyanins (a), expressed in % : $(Abs520) - \frac{Abs520SO2}{Abs520HCl - \frac{5}{3} * Abs520SO2}$

Ionized anthocyanins (mg/L in malvidin 3-O-glucoside) : $\frac{a}{100} * Total\ anthocyanins$

Polymerization index (%) : $\frac{Abs520SO2}{Abs520HCl} * 100$

Total pigments : $Abs520nmHCl$

Polymerized pigments : $Abs520nmSO2$

It is important to specify that 5/3 is used in the total anthocyanin’s formula explains a part of polymeric anthocyanins which is less sensible to pH change and need to be considered in order to allow the estimation of monomeric anthocyanins with a pH < 1. The choice of the factor 5/3 was based upon observation of the properties of polymeric pigment fractions.

2.3.3.2 Individual anthocyanins analysis by HPLC

Monomeric anthocyanins in wine samples were quantified individually through an HPLC system - High performance liquid chromatography – Perkin Elmer Series 200LC - Pump, connector UV/VIS LC-95 and 7725 injector, at 520 nm absorbance, and the column was a Lichrocart 100, RP_{18} col. 5µm, 250mm * 4 mm. Solvent A was 40% formic acid, B was acetonitrile and C was bidistilled water. The program started at 25% A, 6% B and 69% C for 15 minutes, followed by a linear gradient to 25% A, 25.5% B and 49.5% C for 70 minutes. This methodology is based on a paper by Dallas and Laureano (1996).

Although other anthocyanins were present in some wine samples in residual amounts, the peaks considered quantifiable for this study were delphinidin-3-O-monoglucoside, petunidin-3-O-monoglucoside, peonidin-3-O-monoglucoside, malvidin-3-O-

monoglucoside, malvidin-3-O-monoglucoside-acetate and malvidin 3-O-monoglucoside-coumarate.

The injected sample volume was 20 µL and all samples were filtered and analysed in duplicate repetitions.

2.2.4 Condensed tannin evaluation

2.3.4.1 Tannin Power

The tannin power simulates what happens in our mouth because procyanidins bind with the saliva protein that denatures, precipitates and gives us a felt of astringency; for this reason, the method provides that the wine is added with some protein present is BSA (bovine serum albumin) that can bind with procyanidins. The reaction forms a precipitate that is directly imputable to association procyanidins-protein and can be measured by nephelometer (De Freitas and Mateus, 2001).

The method is divided in three steps:

The wine is centrifuged and diluted 1/50 with a wine model solution (12% v/v with 5 g/L of tartaric acid) previously filtrated with cardboard filter of 0,45 µm. The mix is subjected to a nephelometric analysis (1st L/d0)

25 mL of diluted wine is added with 7,5 mL of BSA solution (BSA 0,8 g/L) and shacked in the vortex for 3 or 4 seconds. The wine is stored in a dark ambience for 45 minutes and then is put in nephelometer in order to obtain 2nd L/d1.

In order to know the total result, calculate:

$$\textit{Tannin Power (NTU/mL}^{-1}\textit{)} = \frac{d1-d0}{0.08}$$

2.3.4.2 Proanthocyanins separation according to the polymerization grade

Quantitative and qualitive analysis of proanthocyanins count and their separation according to polymerization grade is made to understand what is happening (in chemical terms) in wines. As example, a high catechins concentrations and a low polymeric proanthocyanins content explain that wine is not evolving (or the evolution is very slow).

In the present study, the different fractions F1, F2, F3 were evaluated, corresponding respectively to the monomeric, oligomeric and polymeric fractions in TNPT 1st and 2nd microvinification, in TNRG 1st and 3rd microvinification and in TNSC 2nd and 3rd microvinification. The analyses were carried out following the Sun *et al*., (1998) method; after having carried out two replicates for each sample, only two of the three microvinfications were selected for each type of sample, i.e. those which gave the most reliable results.

The preparation and the method follow Sun *et al*., (1998) rules reported in the scientific literature, with different passages:

two neutral Sep-Pak (C_{18} Sep-Pak and C_{18} Sep-Pak) cartridges are connected in series, the bigger above the shorter one. The resin is activated with 10 mL of Methanol, 20 mL of distilled water and 15 mL of buffer solution (it can be done with 9,84 g of Na_2HPO_4 + 2,73 g of KH_2PO_4 in 1 L of distilled water in order to obtain pH = 7).

Using a rotatory evaporator (that keep T° around 30°C), 5 mL of wine are dried, and it is defined V1 for the calculations.

In order to elute catechins (F1), oligomeric proanthocyanins (F2), 20 mL of pH=7 buffer is added to the dried wine sample: now it passes through the two Sep-Pak cartridges (in order to eliminate phenolic acids, previously activated); 10 mL of buffer are added again. The cartridges are now dried in N_2 (for 1 hour) and the eluent is thrown away. After 1 hour, with the same dried cartridges, in order to elute catechins and oligomeric catechins, 25 mL of ethyl-acetate is used: the collected fraction is dried out through evaporation (low pressure and T° of 25-30°C). After the evaporation, 3 mL of pH=7 buffer is used to dissolve the dry residue, and it is passed in the ultrasounds machine. A second elution with 15 mL of methanol allows to separate polymeric proanthocyanins and anthocyanins (F3); the solution is dried as well and 5 mL of methanol is added.

The cartridges are re-preconditioned in order to elute F1 + F2 (the cartridges are now dried with N_2 for 1 hour). Catechins (F1) are eluted with 25 mL of ethyl-ether and evaporated, while oligomeric proanthocyanins (F2) are eluted with 15 mL of methanol; both dried residues are dissolved in 5 mL of methanol.

Fractions are detected with vanillin:

Monomeric flavanols (F1) → 2 mL of obtained solution are mixed in two test tubes A and B with 5 mL of sulfuric acid (dilution: 1:3): in the first tube A it is necessary to add 5 mL of methanol (obs. It is the blank, or “zero”) while in the second one B, 5 mL of vanillin

(1% of methanol). The reaction will take around 15 minutes: the absorbance can be read at 500 nm in 20 seconds.

Oligomeric proanthocyanins (F2) → 2 mL of obtained solution are mixed in two test tubes A and B with 5 mL of sulfuric acid (dilution: 1:3). In the first tube A it is necessary to add 5 mL of methanol (obs. It is the blank, or "zero") while in the second one B, 5 mL of vanillin (1% of methanol). The reaction will take around 1 hour and 20 minutes: the absorbance can be read at 500 nm. (The cells are closed with a stopper while reading with the scan mode used in spectrophotometer).

Polymeric proanthocyanins (F3) is equal to (F2).

After obtaining the three absorbance values, the three fractions (F1, F2, F3) can be calculated this way:

$$\left[\frac{mg}{L}\ catechin\right] = \frac{5mL * Abs}{b * V1}$$

Notes:

F1/F2/F3 = monomeric, oligomeric and polymeric fractions defined in the analytical procedure

Abs = the absorbance read at 500 nm for each fraction;

B = slope of the curve F1 (0,0081), F2 (0,0046), F3 (0,0037);

V1 = initial volume of the sample (normally it is used 5 mL).

2.3.4.3 Procyanidins and catechins separation by HPLC

The following method aims at the separation and determination of catechins and proanthocyanins, obtained by extraction and evaluated with high efficiency liquid chromatography (Ricardo da Silva *et al*., 1990).

Due to the absence of the specific standards necessary for the calibration curves construction, which have not been delivered within the study deadlines, in the current experiment, however, only the samples for this analytical procedure have been prepared, while the related data will be published in future related studies.

The samples were prepared starting from the isolation of catechins and procyanidins; it is done by first preparing the Polyamide column, which follows several steps:

The column made of glass has an internal diameter of 2cm, a height of 20cm, and must be vertical throughout the process. Some cotton is placed at the base of the column and with a glass rod and using distilled water, the cotton is well pressed, taking care to leave its upper surface horizontal.

Several layers are then created inside the column: - layer a: 6 g of glass powder with a diameter varying between 200 and 400 µm (OSI Laboratories, France) are placed on the column. With a glass rod and the aid of a pH 7 buffer solution (about 10 mL) all the particles that have adhered to the walls of the column are brought to the bottom, taking care once again that the surface and the upper part are flat and horizontal - layer b: obtained by placing a suspension of 1.2 g of TLC 6 polyamide (Macherey-Nagel, Düren, Germany) in about 20 mL of pH 7 buffer solution, which forms layer c. To avoid the formation of an uneven b/c interface, the polyamide suspension cannot be added directly into the glass powder. The polyamide suspension must be added by dripping it along a glass rod. The stopcock at the base of the column is then opened so that the polyamide deposits slowly on layer b and the buffer solution drips into the underlying flask. After the buffer solution and polyamide have been stirred, this is carefully dropped into the column with the aid of a glass rod, forming layer d. The stopcock is closed after the suspension medium has almost completely drained, only a small layer about 1 cm high should remain on the surface of the column.

The elimination of phenolic acids and other undesirable compounds is carried out by passing 80 ml of buffer solution through the column. This is added carefully, in small quantities (20 mL x 4 times), so as not to disturb the already placed polyamide layer. If the sample is dispersed in a larger volume, the interfering compounds in the subsequent analysis by HPLC are desorbed to the aqueous phase, to be retained again by the polyamide, before the neutral water performs its eluting role (Ricardo-da-Silva *et al*., 1990). The part of the eluent collected up to this point is discarded: it contains the unwanted compounds.

The next step is to separate the catechins and procyanidins:

catechins: the extraction is obtained by passing 25 mL of acetate nitrite 30:70 twice through the column with the previously formed layers, by dripping the collected solution into a small flask placed under the column. The obtained eluent is evaporated at 30°.

procyanidins: the extraction is obtained by passing 25 mL of acetone 75:25 twice in the same column with the previously formed layers, by dripping the collected solution into a small flask placed under the column. The obtained eluent is evaporated at 30°.

The dry residue is dissolved in 1.2 mL of a solution of methanol in double distilled water (50/50 w/v) and the resulting solution is filtered through a methanol resistant 0.45 μm pore filter (Nyflon - syringe filters). The samples obtained are kept in the freezer after the addition of nitrogen, to minimize possible oxidations, in hermetically sealed flasks (1 mL), marked and protected from light.

At the end the separation and the determination of catechins and procyanidins is made by HPLC; to find a compromise between a good resolution of the chromatogram and the shortest analysis time required, two different solvent gradients are used for the separation of the catechins, gradient A and for the separation of the procyanidins, gradient B.

2.4 Sensory analysis

Sensory analysis is intended as an examination of the organoleptic properties of a wine product (ISO standard 5492-2008, Sensory analysis). In the study reported, the panel was made up of 11, 3 female and 8 male tasters, winemakers and experts in degustation, for the red wines evaluation and made up of 7 tasters for white ones; the age of 11 tasters was between 22 – 60. Each of them was called to evaluate the red wines, Touriga Nacional vinified in Portugal (PT), in Rio Grande do Sul (RG) and in Santa Catarina (SC), and the white wines, Alvarinho vinified in Portugal, Rio Grande do Sul and Paranà State (PR).

The parameters taken into consideration were the visual aspect, the nasal perception, and the gustatory perception; attributes were associated with each of the three parameters which the tasters rated on a scale from 0 (less intense) to 5 (more intense). Appearance is evaluated according to terms of colour intensity, clarity and specific colour; the olfactory aroma is represented by indicators, some common, i.e. intensity, persistence and quality of the aroma, while the others of these represent specific scents such as red fruits, tropical fruits, citrus, oak, floral, vegetable and balance. The taste is evaluated by attributes such as acidity, sweetness, bitterness, persistence, astringency, body and finally general balance. By sensory analysis the identification of the most

important descriptors was found, and differences were found in the same varieties TN and AL, grown in Portugal and Brazil. The analysis sheet used by the tasters follows (ANNEXES).

2.5 Statistical analysis

For all the parameters analysed, the results collected were processed with the Excel program; the data obtained as a result of the analyses were reported and the mean and standard deviation were obtained from them to have a better view of the significant differences between samples. For the sensory analysis, the spider diagram and the histogram were also used, having a visual impact and a direct graphical representation of the different characteristics of each sample examined. The data were analysed for their statistical significance, via an analysis of variance (ANOVA), with their significance reported at a 0.05 probability level. The normality of the residues and homoscedasticity were verified through the Shapiro-Wilk test and the Levene test, respectively. When the effect of the factor was significant, two similar pair-wise tests were applied to have double confirmation through two different tests: the Bonferroni post- hoc test, and Tuckey post-hoc test ($P < 0.05$) were used to interpret any significant differences among the mean values. All the analyses were performed using the R software.

3. Results and discussion

3.1 General phenolic composition: total phenols, non-flavonoids and flavonoids

Phenolic compounds play a major role in wine quality, contributing to flavor and colour properties, especially in red wines. The polyphenols are secondary metabolites with diverse chemical structures and functions that are produced during the process of physiological plant growth and/or as a response to certain forms of environmental stress. This large and complex group of secondary metabolites contributes to the characteristics of the grapes and, consequently, of the wines produced from them. These molecules are extracted during the winemaking process, coming from the vine stems and from skins and seeds of the fruit (Ribèreau-Gayon, 1998). The phenolic compounds can be divided into two groups, flavonoids and non-flavonoids. Flavonoids comprise flavanols, anthocyanidins, flavones, and flavanones. The non-flavonoid phenolics consist of cynnamic acids, benzoic acids, and stilbenes. The concentration of the chemical constituents, such as phenolic compounds, in grapes and consequently in the wines can be influenced by factors including grape variety, environmental conditions, production technology, and maceration.

Phenolic compounds can be used to evaluate the chemical authenticity of the geographical origins of the wine. The “terroir” is the name given to the characteristics of the natural locality of grapevine plant in a vineyard. Each “terroir” is influenced by climatic, geological, and soil; each grape produced in a specific “terroir” reflects the locality in its chemical composition.

The reported paragraph highlights the differences between the total phenols, non-flavonoids and flavonoids of the Alvarinho variety grown in Portugal and in the Brazilian regions of Rio Grande do Sul and Paranà State. The presence of phenolic compounds is very limited compared to red wines, as contact with the solid parts occurs only in the pre-fermentation phase. White wines mostly contain benzoic and cinnamic acids, catechins, procyanidins and flavonols. The analyses conducted on ALPT, ALRG, ALPR are reported in figure 8. The samples of the Brazilian microvinifications showed higher values than the microvinifications of the wines obtained from Portuguese territories.

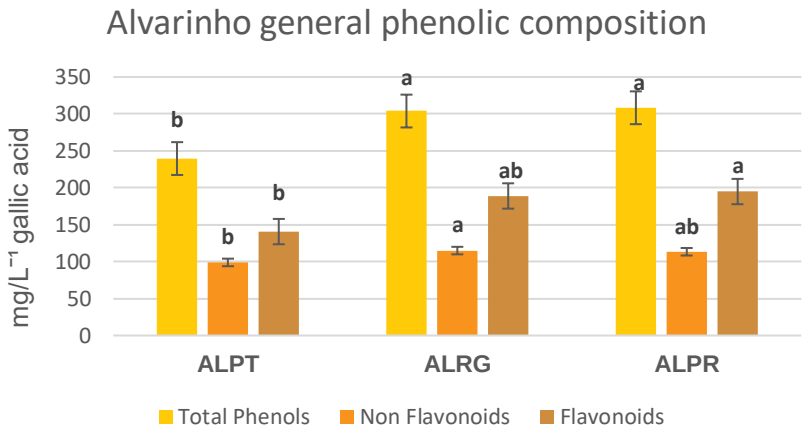


***Figure 8**. General phenolic composition in Alvarinho cultivated in Portugal, Rio Grande do Sul and Paraná State*

*Legend: **ALPT**:Alvarinho Portugal, **ALRG**:Alvarinho Rio Grande do Sul, **ALPR:** Alvarinho Paraná State; mean values with the same letter indicate no statistically significant differences (Tukey's test at p<0.05).*

As can be seen from the graph (Figure 8.), the different letters indicate significant differences between ALPT and the Brazilian samples, ALRG and ALPR, between which no notable differences emerge.

The same analyses were also applied to the Touriga Nacional samples. As the figure 9 shows, in agreement with what the averages found of the microvinifications, in the TNPT samples there is a higher concentration of total phenols and flavonoids than in the microvinifications of TNRG and TNSC. The maximum concentration in terms of total phenols and flavonoids detected dates to TNPT samples, while the minimum concentration of the same is attributed to TNRG ones. In the case of non-flavonoid compounds, the results are opposite. From the figure 9 it can be seen that the latter are greater in Brazilian microvinifications than in Portuguese ones; the non-flavonoid forms are benzoic and cinnamic acids. They differ in the degree and nature of the substituents of the benzene ring. The free forms are mainly present in red wines, due to the hydrolysis of their combined forms and the degradation reaction of more complex molecules, in particular anthocyanins, under the action of heat. The lower concentration of these compounds in TNPT wines can be justified by the fact that reactions involving non-flavonoids occurred within these samples. Finally, the concentration of flavonoids was significantly higher in TNPT than in Brazilian wines.

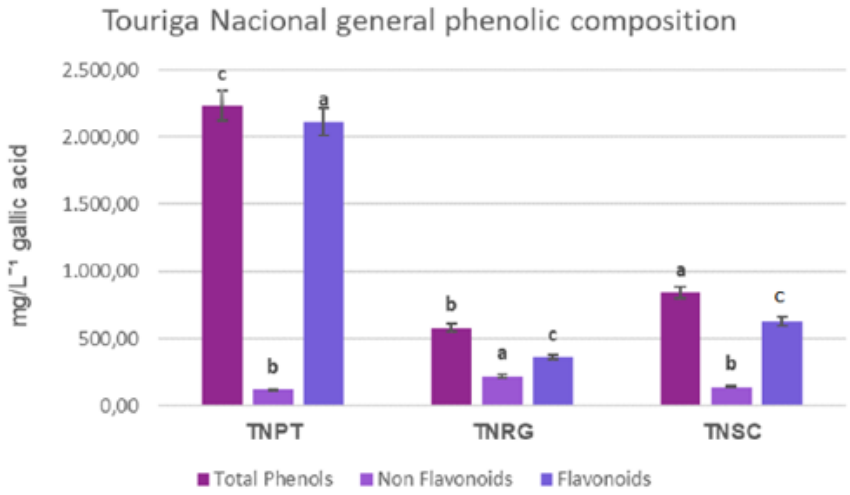


Figure 9. *General phenolic composition of Touriga Nacional cultivated in Portugal, Rio Grande do Sul and Santa Catarina*

Legend: ***TNPT****: Touriga Nacional Portugal,* ***TNRG****: Touriga Nacional Rio Grande do Sul,* ***TNSC:*** *Touriga Nacional Santa Catarina; mean values with the same letter indicate no statistically significant differences (Tukey's test at p<0.05).*

The differences highlighted in the figure 9, which occur between the results of the different TNPT, TNRG and TNSC microvinfications are proof of the fact that these values reflect the terroir of the different production regions; many factors can affect these results like storage temperature is the major factor contributing to the changes in phenolic acid content in grapes. Degradation of phenolic acids, such as gallic acid, can be influenced by temperature and elevated rainfall during the maturation phase of some grape cultivars cultivated in Brazil. Therefore, the reduction of total phenols in Brazilian wines may have been influenced by climatic factors such as temperature, solar radiation and rainfall during the maturation phase of the grapes, before being submitted to fermentation for wine production (Belmiro *et al*., 2017).

3.2 Chromatic characterization

3.2.1 Colour intensity and hue

In agreement with the previous data, the analyses also carried out in the order to determine the colour intensity and hue, showed divergent results between microvinifications of TNPT, TNRG and TNSC. The figure 10 shows the differences found following the analysis of the colour intensity in the Touriga Nacional variety grown in Portugal, in Rio Grande do Sul and in Santa Catarina State. As can be seen from the

graph (Figure 10.), the intensity was higher in the TNPT samples than in the same variety grown in Brazil. Using the same analysis method (OIV Method MA-AS2-07B), the colour tone was also evaluated: from figure 11 it can be seen that no particularly significant differences were found with regard to this parameter. However, the letters indicate that the colour tone evaluated in TNPT and TNSC is almost identical, while that measured in TNRG is lower, differing significantly.

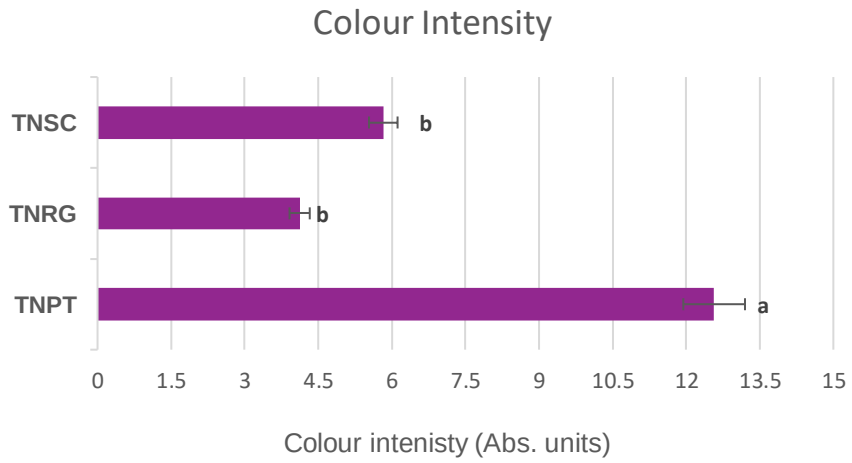


***Figure 10**. Colour intensity of Touriga Nacional from Portugal, Rio Grande do Sul and Santa Catarina State*

*Legend: **TNPT**: Touriga Nacional Portugal, **TNRG**: Touriga Nacional Rio Grande do Sul, **TNSC:** Touriga Nacional Santa Catarina; Mean values with the same letter indicate no statistically significant differences (Tukey's test at p<0.05)*

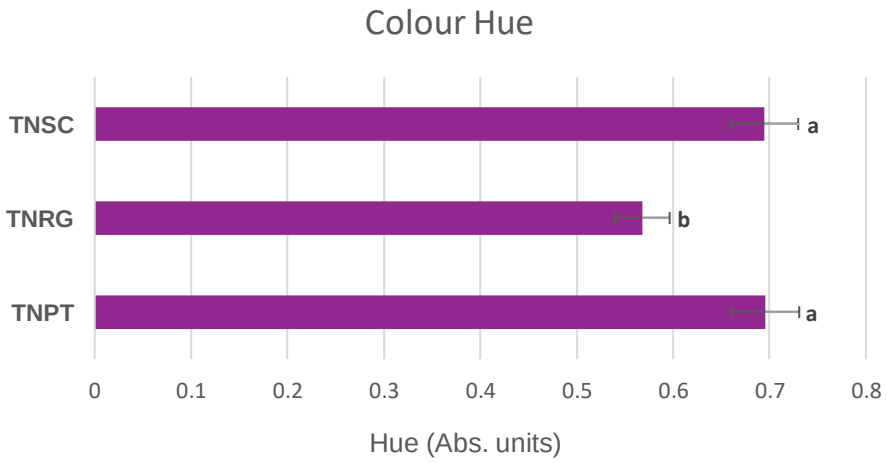


***Figure 11**. Colour hue of Touriga Nacional from Portugal, Rio Grande do Sul and Santa Catarina State*

*Legend: **TNPT**: Touriga Nacional Portugal, **TNRG**: Touriga Nacional Rio Grande do Sul, **TNSC:** Touriga Nacional Santa Catarina; Mean values with the same letter indicate no statistically significant differences (Tukey's test at p<0.05)*

3.2.2 Colorimetric coordinates

3.2.2.1 CIE/Lab Alvarinho

In the current study, the colour of Alvarinho, ALPT, ALRG, ALPR white wines is evaluated by CIE/Lab analysis (Table 4). In white wines the presence of phenolic compounds is very limited, compared to red wines, as contact with the solid parts occurs only in pre-fermentation maceration. Musts and white wines contain benzoic and cinnamic acids, catechins, procyanidins and flavonols. In dry white wines the total phenolic content represents from 50 to 250 mg/L, i.e. less than 10% of that of red wines. The phenolic contribution in the whites is mainly linked to the oxidative browning of the wines.

***Table 4**. Alvarinho CIE/Lab results: general means and standard deviation values*

Variable	**Wine**			
	ALPT	ALRG	ALPR	
L*	95,21 ± 0,323 b	95,06 ± 0,037 b	96,73 ± 0,430 a	*
a*	-0,3235 ± 0,053 a	-1,0775 ± 0,018 b	-1,9366 ± 0,011 c	***
b*	9,817 ± 0,094 c	13,91 ± 0,030 b	16,37 ± 0,042 a	***
C*	9,826 ± 0,094 b	16,98 ± 0,031 a	16,5 ± 0,044 a	***
H*	-87,987 ± 0,301 b	-86,358 ± 0,056 b	-83,232 ± 0,059 a	***
H*C	272,00 ± 0,301 b	273,63 ± 0,0,57 b	276,76 ± 0,059 a	***

*Legend: **ALPT**: Alvarinho Portugal, **ALRG**: Alvarinho Rio Grande do Sul, **ALPR:** Alvarinho Paraná State; mean values with the same letter indicate no statistically significant differences (Tukey's test at p<0.05); p-value (*not very significant/ **significant/ ***highly significant); Clarity = **L***, Red-green colour = **a***, Yellow – blue colour = **b***, Chroma = **C***, Hue = **H***, H*correction = **H* C**.*

As can be seen from the table 4, the values of L* and H* are similar in the 3 microvinifications of ALPT, ALRG and ALSC and they are not significantly different even some differences emerged during the analyses between the three types respectively. From the analyses it emerges that in ALPT lower results were obtained for the parameters a*; no significant differences emerged between the samples obtained from Brazilian cultures. Even for the b* value, lower values were found in ALPT microvinifications, and lower values in the ALRG as well compared to the other ALPR samples. Finally, also for C*, lower results emerge for the ALPT wines compared to those of ALRG and ALPR, between which there are no significant differences. Finally, for the parameter H*C there were no significant differences between ALPT and ALRG; as can be seen from the table 4, the letters showed a significant difference in ALPR.

The divergences emerged between the samples obtained from cultivations in Portugal, in Rio grande do Sul and in Paraná State, involving red green colour component a*, blue

yellow colour component b*, and the chroma C*, could be attributed to the different ripening conditions and to the climatic conditions under which it occurred. Colour starts to change in the early stages of winemaking via enzymatic reactions (Ribéreau-Gayon *et al*., 2006) caused by the active polyphenol oxidase, in which hydroxycinnamic esters play an important role. After fermentation, non-enzymatic oxidation usually occurs, which give rise to a brown colour progressively replacing the initial colour (generally pale-yellow) of white wines (Li *et al*., 2008; Oliviera *et al*., 2011; Pati *et al*., 2006; Pati *et al*., 2014).

The most important constituents in white wines which are able to participate in oxidation reactions are hydroxycinnamates and flavanols. In particular, the oxidation of ortho-dihydroxyphenolic compounds (such as (+)-catechin and (-)-epicatechin) and hydroxycinnamic acids results in the formation of yellow-brown compounds due to the polymerization of ortho-quinones (Cheynier *et al*., 1989; Guyot *et al*., 1996). Indeed, studies on wine model solutions have confirmed the formation of two types of yellow pigments, xanthylium salt pigments and ethyl- ester of xanthylium salts, showing visible absorption maxima at 440 and 460 nm respectively, both of which are derived from flavanol oxidation and polymerization (Es-Safi *et al.*, 2000). Other constituents of the wine, such as SO_2 and ascorbic acid, are also of high importance in polyphenol oxidation; when added to wine, they are able to reduce the ortho-quinones (Singleton, 1987). Moreover, the presence of polysaccharides (released by inactivated dry yeasts) can have a positive effect on the colour of the wines. This colour protection could be due to the interaction of polysaccharides and certain phenolic compounds which are easily-oxidizable (hydroxycinnamic tartaric esters), as well as to reduced oxidation through higher oxygen consumption (Del Barrio Galan *et al*., 2018).

Other parameters, both intrinsic and environmental, are key factors in determining the extent of browning oxidation in white wine. In addition to the effect of grape variety and the degree of maturity at time of harvest, temperature, oxygen content and pH increase the browning rate (as measured by the change in optical density at a wavelength of 420 nm) (Karbowiak *et al*., 2010). For white wines, colour change is more dependent on light exposure than oxygen concentration at 20°C, whereas at 45°C their respective effects become equal (Cartagena *et al*., 1994). High pH and high temperature are also found to increase the browning of wine. An increase in pH increases the concentration of phenolate ions relative to the phenol form, thus increasing oxidation rates by about nine times when pH is between 3 and 4 (Singleton, 1987). However, it should be noted that the different factors (temperature, oxygen, pH, light) involved in the oxidation of white

wines during storage have an effect on wine oxidation rate as a whole, and the isolated effects of each parameter are very difficult to study (Karbowiak *et al.*, 2010).

According to what emerges from these studies, it is therefore possible to hypothesize that the colour differences obtained in terms of brightness, saturation and chroma between the microvinifications obtained in Portugal and Brazil, are attributable to all these factors: the hotter and more humid climate of the Brazilian regions during the harvest and the winemaking processes, they would therefore have had a greater impact on the colour of the grapes and on the reactions triggered.

3.2.2.2 CIE/Lab and Copygmentation of Touriga Nacional

The colour of red wines is attributed to anthocyanins, the pigments of the grapes. They are essentially found in the skin in increasing quantities, more when the vine is at the end of the vegetative cycle. As in the case of Alvarinho, the colour of these pigments is a function of the composition of the medium (pH, SO_2) and depends on their molecular structure and on that of the substances present together with them. The content of these compounds varies significantly with the age of the wines and with the nature of the variety from which they derive. After vinification, they decrease rapidly during refinement and ageing.

Most of these pigments, however, associate or condense with wine tannins to form another class of more stable colour molecules responsible for colour. Another relatively small part of anthocyanins disappears, due to the degradation of external agents (temperature, light and oxygen..), and by precipitation as colloidal colouring matter.

In the present study, the colour of Touriga Nacional wines vinified in Portugal and in the Brazilian regions of Rio Grande do Sul and Santa Catarina was evaluated through the data obtained from the analysis of the following parameters: luminosity L*, red green colour component a*, blue yellow colour component b*, chroma C*, brightness angle H*, by CIE/Lab analysis (Table 5).

In agreement with what is reported in table 5, the L* values found during the analyses of the Touriga Nacional samples are higher in TNRG and TNSC than those of TNPT; in accordance with what is expected in a red wine, the red-green components (a*) predominate over the yellow-blue ones (b*): the values are significantly higher in the 3 microvinfications of TNPT than in the Brazilian ones of TNRG and TNSC, among which

no significant difference is found. In agreement with what has just been highlighted, also the C* detected in the TNPT samples is significantly higher than that of TRG and TNSC.

***Table 5**. Touriga Nacional CIE/Lab results: general means and standard deviation values*

Variable	Wine			
	TNPT	TNRG	TNSC	
L*	63,71 ± 0,163 c	85,28 ± 0,159 a	80,28 ± 0,213 b	***
a*	35,78 ± 0,666 a	15,60 ± 0,124 b	16,83 ± 0,027 b	***
b*	0,666 ± 0,204 a	-0,925 ± 0,025 b	0,28 ± 0,111 a	***
C*	35,79 ± 0,172 a	15,55 ± 0,125 b	17,58 ± 1,057 b	***
H*	0,964 ± 0,084 a	-3,413 ± 0,058 b	0,958 ± 0,382 a	***
H*C	120,95 ± 0,090 b	356,58 ± 0,057 a	0,958 ± 0,385 c	***

*Legend: **TNPT**: Touriga Nacional Portugal, **TNRG**: Touriga Nacional Rio Grande do Sul, **TNSC:** Touriga Nacional Santa Catarina; mean values with the same letter indicate no statistically significant differences (Tukey's test at p<0.05); p-value (*not very significant/ **significant/ ***highly significant); Clarity = **L***, Red-green colour = **a***, Yellow – blue colour = **b***, Chroma = **C***, Hue = **H***, H*correction = **H* C**.*

In relation to the CIE/Lab, also the parameters shown in figure 12, show the differences between Portuguese and Brazilian wines. Copygmentation concerns the formation of complexes, both between different forms of anthocyanins and between anthocyanins and other usually colorless phenolic compounds. This phenomenon depends on various factors: nature, concentration of anthocyanins, type and content of pigments, pH, temperature and nature of the solvent. This phenomenon is generally less favored in wine than in grape juice, since with the appearance of ethanol, the bond that intervenes in copygmentation is broken. In wine, a new copygmentation can be observed due to higher tannins than anthocyanins: it leads to an increase in anthocyanin colouring and a slight shift of lambda max towards blue. From the copygmentation figure trend (Figure 12.), it can be seen that the microvinfications of TNPT have much higher significant values (in the order of about 200 a.u) than those of TNRG (in the order of about 45 a.u) and TNSC (in the order of 55 a.u).

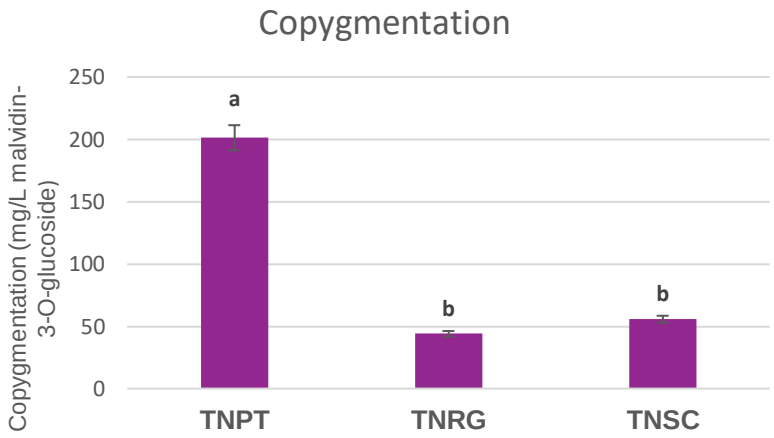


***Figure 12**. Copygmentation features of Touriga Nacional from Portugal, Rio Grande do Sul and Santa Catarina*

*Legend: **TNPT**: Touriga Nacional Portugal, **TNRG**: Touriga Nacional Rio Grande do Sul, **TNSC:** Touriga Nacional Santa Catarina; Mean values with the same letter indicate no statistically significant differences (Tukey's test at p<0.05)*

3.3 Pigment evaluation and characterization

3.3.1 Anthocyanins and pigment contents

The results obtained from the spectrophotometry method for total and ionized anthocyanins, as well as ionization degree, polymerization index, total pigments and polymerized pigments are shown in figure 13.

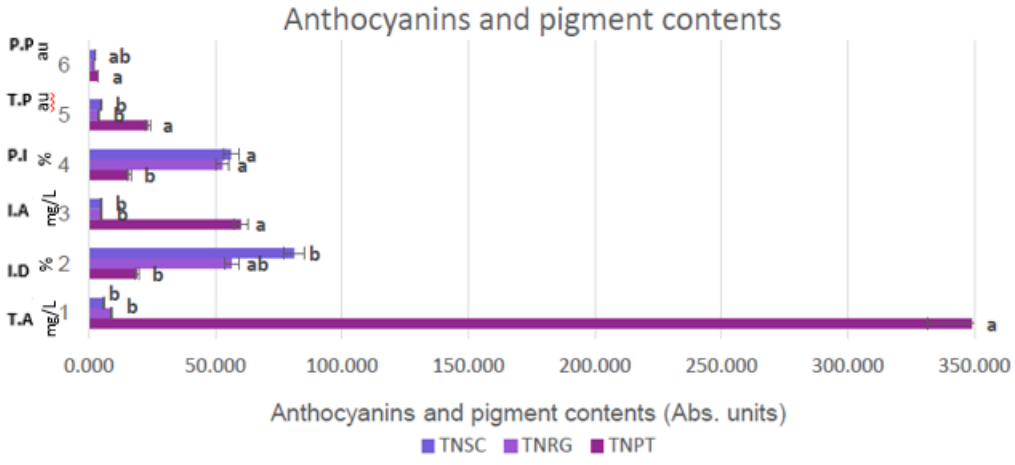


***Figure 13**. Anthocyanins and pigment contents of Touriga Nacional from Portugal, Rio Grande do Sul and Santa Catarina*

*Legend: **TNPT**: Touriga Nacional Portugal, **TNRG**: Touriga Nacional Rio Grande do Sul, **TNSC:** Touriga Nacional Santa Catarina; Mean values with the same letter indicate no statistically significant differences (Tukey's test at p<0.05); **T.A:** Total anthocyanins (mg/L); **I.D**: Ionization degree (%); **I.A**: Ionized anthocyanins (mg/L); **P.I**: Polymerization index (%); **T.P**: Total pigments (a.u); **P.P**: Polymerized pigments (a.u).*

The highest concentrations of total anthocyanins were in the order of a mean of 349,13 mg/L of malvidin in TNPT; as shown in the figure 13, and more clearly also from figure 14 for total anthocyanins, a highly significant difference was found between the TNPT samples and the Brazilian wines, TNRG and TNSC; there was no significant difference between these last ones. Notable differences also emerged proportionately in the concentrations of ionized anthocyanins and total pigments: the highest values were found for the Portuguese microvinifications, significantly higher than the data emerging for the Brazilian microvinification (Figure 15).

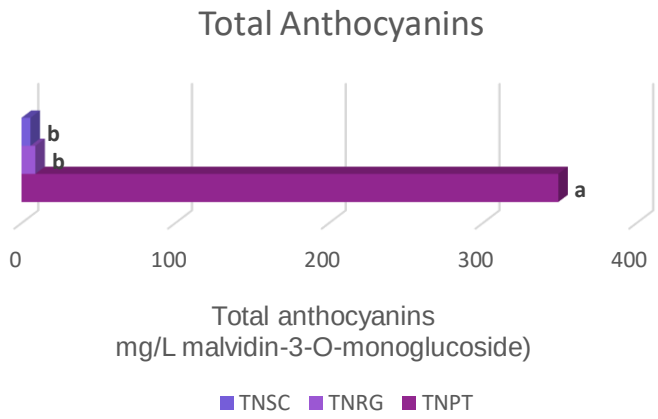


***Figure 14**. Total anthocyanins of Touriga Nacional from Portugal, Rio Grande do Sul and Santa Catarina*

*Legend: **TNPT**: Touriga Nacional Portugal, **TNRG**: Touriga Nacional Rio Grande do Sul, **TNSC:** Touriga Nacional Santa Catarina; Mean values with the same letter indicate no statistically significant differences (Tukey's test at p<0.05);*

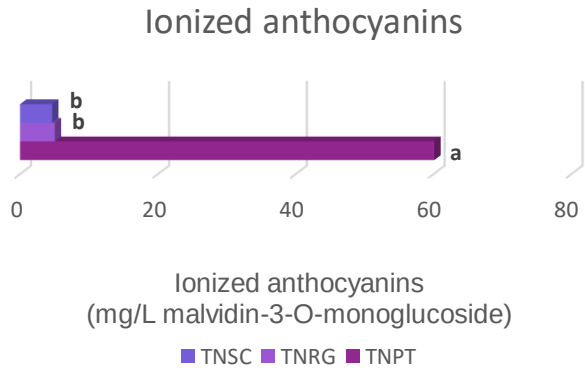


***Figure 15**. Ionized anthocyanins and total pigments in Touriga Nacional from Portugal, Rio Grande do Sul and Santa Catarina*

*Legend: **TNPT**: Touriga Nacional Portugal, **TNRG**: Touriga Nacional Rio Grande do Sul, **TNSC:** Touriga Nacional Santa Catarina; Mean values with the same letter indicate no statistically significant differences (Tukey's test at p<0.05)*

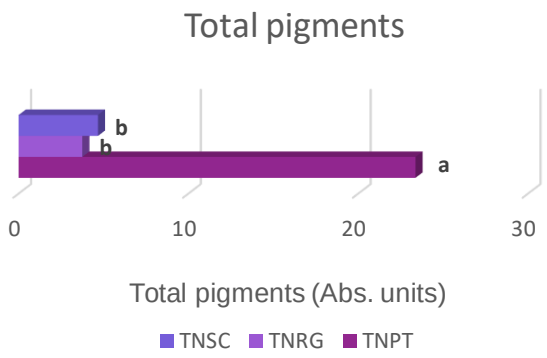


***Figure 16**. Ionized anthocyanins and total pigments in Touriga Nacional from Portugal, Rio Grande do Sul and Santa Catarina*

*Legend: **TNPT**: Touriga Nacional Portugal, **TNRG**: Touriga Nacional Rio Grande do Sul, **TNSC:** Touriga Nacional Santa Catarina; Mean values with the same letter indicate no statistically significant differences (Tukey's test at p<0.05)*

The results relating to the degree of ionization and the polymerization index expressed as a percentage, as shown in figure 16, are inversely proportional to the previous data: observing the averages of these parameters of the Brazilian microvinfications of TNRG and TNSC, they are significantly greater than those of TNPT.

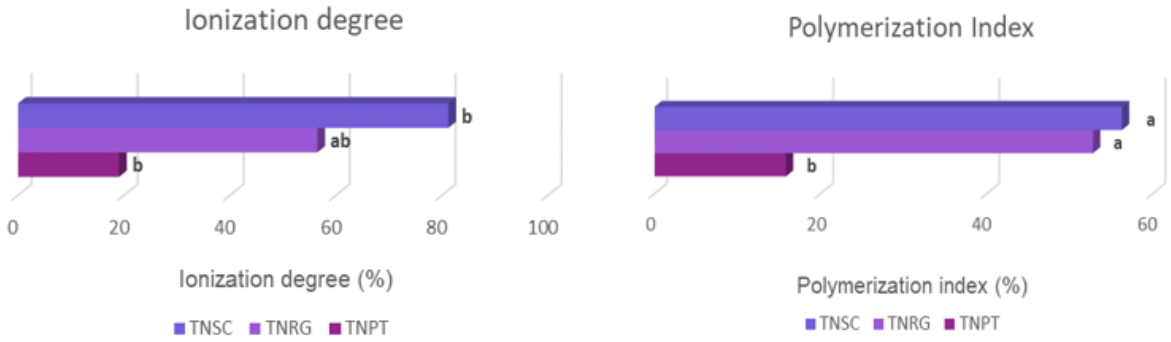


***Figure 17**. Ionization degree and polymerization index of Touriga Nacional from Portugal, Rio Grande do Sul and Santa Catarina*

*Legend: **TNPT**: Touriga Nacional Portugal, **TNRG**: Touriga Nacional Rio Grande do Sul, **TNSC:** Touriga Nacional Santa Catarina; Mean values with the same letter indicate no statistically significant differences (Tukey's test at p<0.05)*

The ionization index allows to define the percentage of free and combined anthocyanins that are found in the coloured form in the wine; as the anthocyanins combine with the tannins, the percentage increases as the new pigments are much less sensitive to discoloration by the combined action of pH and SO_2.

The last parameter evaluated was that of polymeric pigments: also in this case, as can be seen from figure 17, the highest values were found in TNPT wines compared to Brazilian wines. The figure highlights a significant difference between TNPT and TNRG

showing lower values; a less significant difference was instead highlighted between TNPT and TNSC.

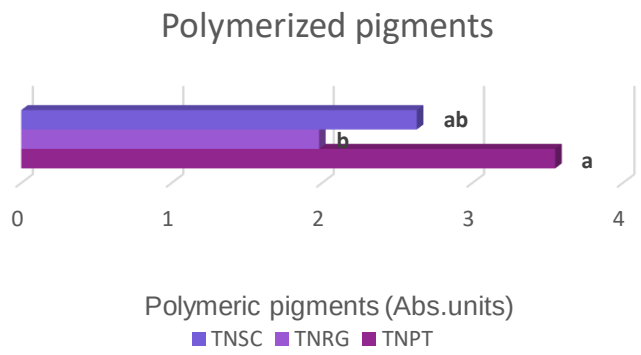


***Figure 18**. Polymerized pigments of Touriga Nacional from Portugal, Rio Grande do Sul and Santa Catarina*

*Legend: **TNPT**: Touriga Nacional Portugal, **TNRG**: Touriga Nacional Rio Grande do Sul, **TNSC:** Touriga Nacional Santa Catarina; Mean values with the same letter indicate no statistically significant differences (Tukey's test at $p<0.05$)*

The differences that emerged in these results could be connected to a different extractability of tannins and anthocyanins, or to changes in the structure and composition of them, factors related to the exposure of the bunches and the temperature of the cultivation environment. In relation to this, some studies have been conducted which suggest that temperature has a greater effect on light: the anthocyanin concentration is higher when the temperature is constant and balanced (Buttrose *et al*., 1971). The increase in temperature in the plant, either through the direct heat brought about by solar incidence or by the increase in air temperature, on the one hand can increase the metabolic processes in the plant which are associated with an increase in development and accumulation of metabolites (Hawker, 1982; Jones, 1992). However, at high temperatures many metabolic processes are stopped or are significantly reduced. Gladstones (1992) suggests that pigment formation and the optimal physiological ripening of grapes for the synthesis of colour and aroma compounds take place between 20°C and 22°C. When the day temperature is high, low night temperatures are necessary to ensure a low pH and high natural acidity (Jackson & Lombard, 1993). Mori *et al*., (2005) found that metabolic pathways are altered when the ambient temperature reaches 30°C.

Furthermore, humidity also plays a fundamental role: by lowering the vapor pressure, transpiration and photosynthesis decrease, reducing growth and consequently the accumulation of phenolic compounds (Lamb, 1987). The temperature of the bunch is

therefore fundamental for a greater or lesser synthesis of the compounds analysed: specifically in warm- climate viticulture, this frequently reaches a level which can inhibit the synthesis of anthocyanins. Environmental factors, geographical conditions, and the specific conditions that occur during each harvest can have a different impact on the concentration of phenolic compounds and their distribution.

Some authors have reported that climatic conditions characterized by high concentrations of rainfall before flowering followed by a season characterized by warm weather during grape ripening, can cause low anthocyanin synthesis (Downey and Hanlin, 2009). The hot-humid climate of the Brazilian regions may be in response to the low level of synthesis and faster degradation of these compounds. As reported below in the paragraph of tannin power (3.4.1), the synthesis of these compounds is also influenced by the type of soil; an excessive humidity index even in the soil induces an osmotic stress in the plant which decreases the synthesis of colouring compounds.

Finally, the factor of altitude can also have a significant impact on the synthesis of pigments: some studies have proved that increasing altitude can also increase the synthesis of anthocyanins; the altitude of the Dão Region, where TNPT were grown, may have positively influenced their synthesis. The Santa Catarina region has a high altitude; however, this does not seem to have significantly influenced the wines examined in terms of anthocyanin development (Oliveira *et al*., 2006).

3.3.2 Individual anthocyanins analysis by HPLC

In agreement with the previously analysed data, also the analysis of monomeric anthocyanins showed differences between the Portuguese and Brazilian samples. Through HPLC analysis, the results of individual anthocyanin concentration and distribution were obtained. The analyses were carried out on the samples of the three microvinifications of TNPT, on the first and third microvinification of TNRG and finally on the second and third of TNSC. The monoglucoside forms of delphinifin, petunidin, peonidin and malvidin were evaluated (Figure 18.). Of the same anthocyanidins, the content of the acetylated (Figure 19.) and coumaroyl derivative (Figure 20.) forms was evaluated.

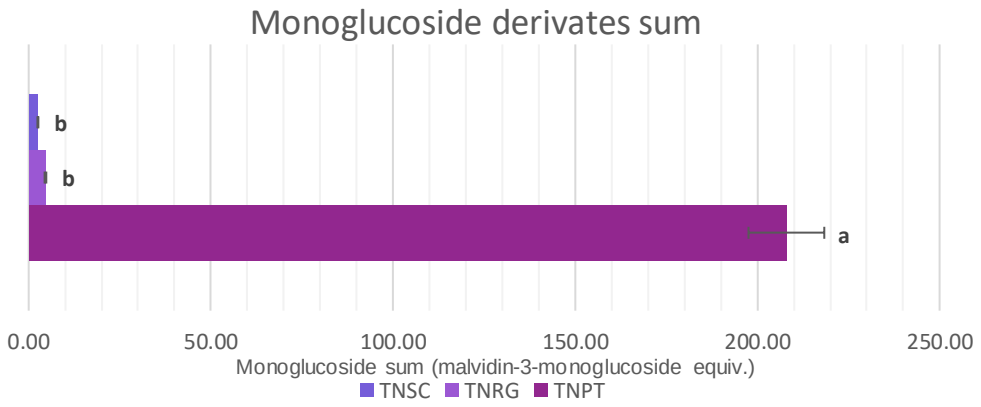


***Figure 19**. Monoglucoside sum results of Touriga Nacional from Portugal, Rio Grande do Sul and Santa Catarina*

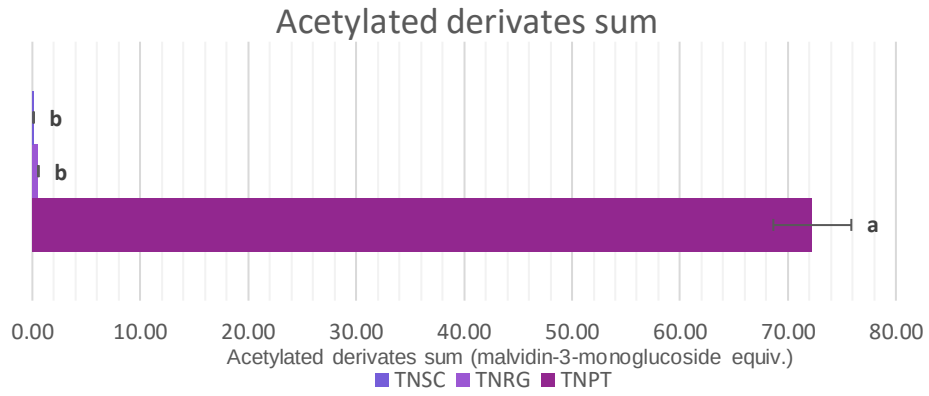


***Figure 20.** Acetylated derivates sum results of Touriga Nacional from Portugal, Rio Grande do Sul and Santa Catarina*

*Legend: **TNPT**: Touriga Nacional Portugal, **TNRG**: Touriga Nacional Rio Grande do Sul, **TNSC:** Touriga Nacional Santa Catarina; Mean values with the same letter indicate no statistically significant differences (Tukey's test at p<0.05)*

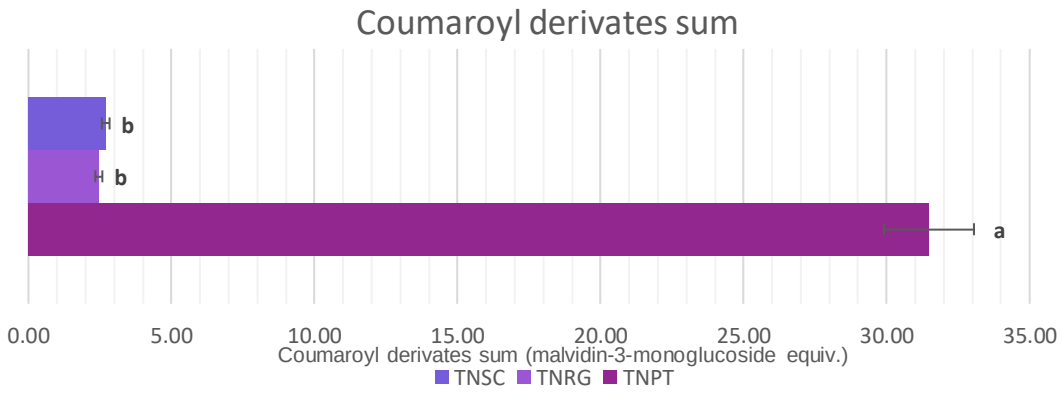


***Figure 21**. Coumaroyl derivates sum results of Touriga Nacional from Portugal, Rio Grande do Sul and Santa Catarina*

*Legend: **TNPT**: Touriga Nacional Portugal, **TNRG**: Touriga Nacional Rio Grande do Sul, **TNSC:** Touriga Nacional Santa Catarina; Mean values with the same letter indicate no statistically significant differences (Tukey's test at p<0.05)*

As shown in the figures, the highest concentration of anthocyanidins in all three forms considered is always significantly higher in TNPT, and almost entirely absent in TNRG and TNSC in the monoglucoside sum and acetylated forms.

Figure 21 represents the sum total of the concentrations of all forms of monomeric anthocyanidins considered in the analyses: the difference between Touriga Nacional grown in Portugal and in the Brazilian regions emerges significantly.

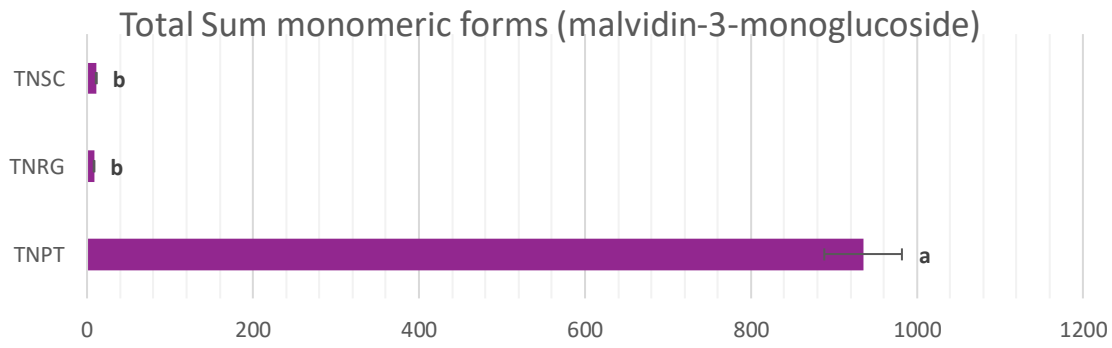


***Figure 22**. Total sum monomeric forms results of Toutiga Nacional from Portugal, Rio Grande do Sul and Santa Catarina*

*Legend: **TNPT**: Touriga Nacional Portugal, **TNRG**: Touriga Nacional Rio Grande do Sul, **TNSC:** Touriga Nacional Santa Catarina; Mean values with the same letter indicate no statistically significant differences (Tukey's test at $p<0.05$)*

The distribution of all forms is shown in figure 22. In agreement with what is shown in the previous graphs, all forms are present in TNPT, in higher or lower concentrations depending on the type of anthocyanidin. Malvidin-3-glucoside and malvidinin-3 p-coumaroylglucoside are present in TNRG and TNSC; the other forms were found to be absent or at concentrations close to zero.

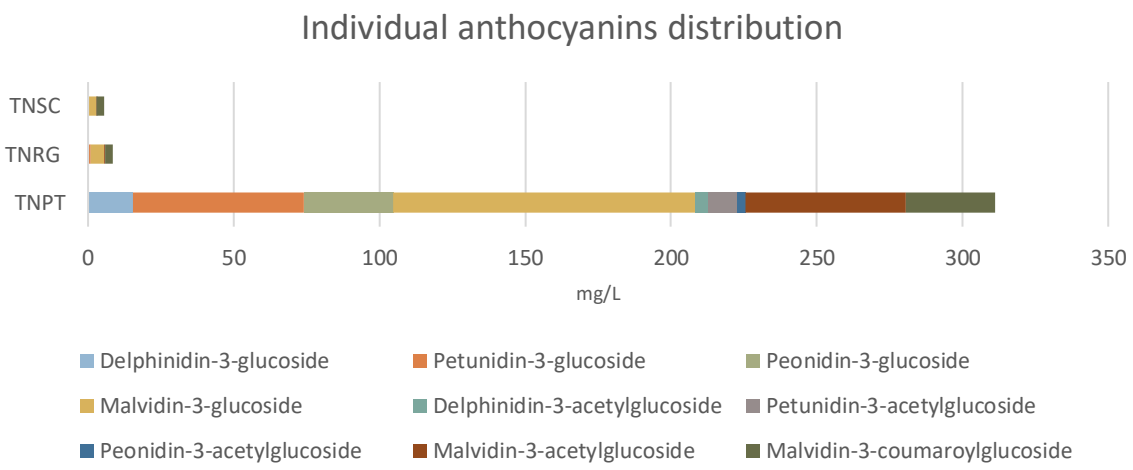


Figure 23. *Individual anthocyanins distribution of Touriga Nacional from Portugal, Rio Grande do Sul and Santa Catarina*

Legend: ***TNPT****: Touriga Nacional Portugal,* ***TNRG****: Touriga Nacional Rio Grande do Sul,* ***TNSC:*** *Touriga Nacional Santa Catarina*

It is observed in figure 22 that there is a variation of anthocyanins between regions, being able to be an indicator of environmental conditions. The group of monomeric anthocyanins in their 3-O-glucoside form (delphinidin, peonidin, petunidin and malvidin) obtained higher concentrations in wines from Portugal. Furthermore, the presence of 3-O-malvidin and 3-malvidin-coumaroylglucoside as the only monomeric anthocyanins in TNSC and TNRG, could be linked to climatic conditions, such as hot temperature and high altitude in the case of Santa Catarina, that collaborate with the enzymatic activity coumaroyl transferase, improving their stability and presence. However, there are no studies in the literature that proves a direct relationship between synthesis of coumarylated anthocyanins and the altitude (Oliveira *et al.*, 2018).

3.4 Condensed tannin evaluation

3.4.1 Tannin power

The tannins of the skin and seeds accumulate starting from veraison: while the tannins of the skin grow during maturation, the tannins of the seeds tend to decrease; their decrease varies according to the ripening but also according to the variety (Glories, 1986). There are many factors reputed to affect flavonoid biosynthesis in plants,

including light, temperature, altitude, soil type, water, nutritional status, microbial interactions, plant growth regulators, and various developmental processes. Characters as the parent material and the age of the soil largely determine the micronutrient pool, structure, and texture of soil, which have a significant effect on the plant growth (Rusell, 1961). However, the major consequence of soil type is the capacity of the soil to hold water while remaining well drained. Some reports suggest water deficit increases tannin and anthocyanin content in grapes (Dry *et al*., 1998). Some research suggests that while excessive water decreases tannin content (Kennedy *et al*., 2000), water deficit has a little or no effect on tannin accumulation in the grape berry. Rather, the primary effect of the water deficit is to decrease berry size and thus change the ratio of skin to total berry weight and therefore tannin concentration in the berry. What has just been reported is demonstrated in the figure 23.

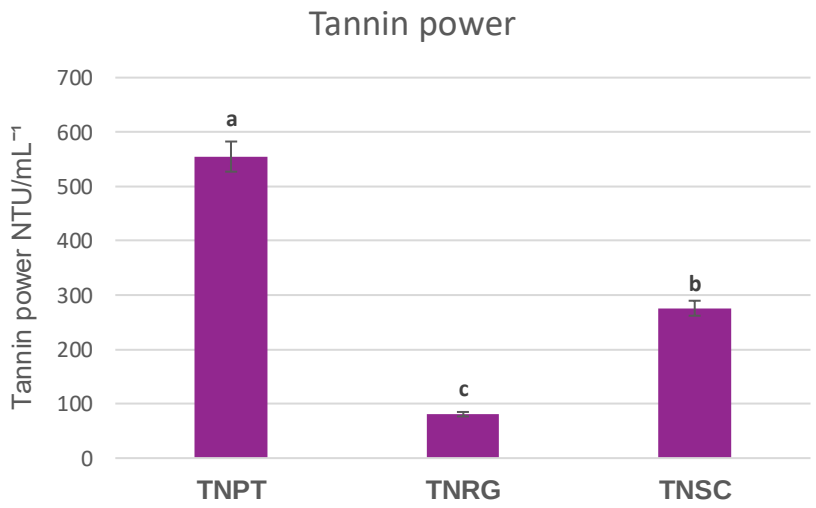


***Figure 24**. Tannin power results of Touriga Nacional from Portugal, Rio Grande do Sul and Santa Catarina*

*Legend: **TNPT**: Touriga Nacional Portugal, **TNRG**: Touriga Nacional Rio Grande do Sul, **TNSC:** Touriga Nacional Santa Catarina; Mean values with the same letter indicate no statistically significant differences (Tukey's test at p<0.05)*

The trend of the columns represented in figure 23 clearly shows how the values relating to the tannin power are higher in the TNPT wines, compared to the data obtained from the TNSC and TNRG ones. In this case the statistical analysis revealed significant differences between the three types of Touriga Nacional, each indicated in fact with a different letter (a, b, c).

In accordance with the climatic conditions reported in the introduction for Dão Region, Rio Grande do Sul and Santa Catarina, the tannin power is higher in the three microvinifications of TNPT coming from a region with a temperate climate and alternating rain and dry conditions. Moderate soil water and osmotic stress allowed TNPT to accumulate a higher concentration of tannins. The effect of altitude on grape berry development and composition has also been examined: flavan-3-ol monomer, dimer, and total proanthocyanin content in the skin of grapes decreases with increasing altitude, seed monomers and proanthocyanins decrease as well (Mateus *et al*., 2001).

The high altitude of the Santa Catarina region reduced the tannin content in TNSC, relative to TNPT, to an average value between 260 and 290 NTU. However, the lower tannin power is that measured in the TNRG samples, where the sub-tropical climate and more frequent rainfall increase the osmotic stress of the soil, disfavoring the synthesis of tannins. Studies have also been conducted on the effect of exposure to light on the increase and decrease in the concentration of tannins in grapes. As reported by Spayd *et al*., (2002), the most exposed fruits have a higher content of tannins, compared to the shaded bunches.

The results obtained by TNPT, TNRG, TNSC can therefore also be associated with a greater or lesser exposure to solar radiation in the different regions and/or exposure of the plants in the vineyards; a greater or lesser synthesis of these compounds depending on the light is also directly propitious to the agronomic practices adopted in the respective vineyards and production areas: an intense defoliation of the foliage exposes the bunches to the light favouring the synthesis, while if the defoliation is limited, decreasing the synthesizing ability (Oliveira *et al*., 2018).

3.4.2 Proanthocyanins separation according to the polymerization grade

In the present study, according to the previous data, also in this case, the table 6, shows that the highest values are attributable to the microvinifications of TNPT: the largest fraction is the polymeric one, followed by the oligomeric and finally the monomeric one.

***Table 6**. Means and standard deviations results of proanthocyanins fractions of Touriga Nacional from Portugal, Rio Grande do Sul and Santa Catarina*

Fraction	**Wine**			
	TNPT	TNRG	TNSC	
Monomeric F1 (mg/L)	27,4 ± 0,3 a	14,4 ± 9,0 b	4,9 ± 0,8 c	**
Oligomeric F2 (mg/L)	91,3 ± 6,1 a	5,9 ± 0.7 b	2,7 ± 0,7 b	***
Polymeric F3 (mg/L)	413,1 ± 118,4 a	16,8 ± 0,9 b	28,3 ± 1,9 b	*

*Legend: **TNPT**: Touriga Nacional Portugal, **TNRG**: Touriga Nacional Rio Grande do Sul, **TNSC:** Touriga Nacional Santa Catarina; mean values with the same letter indicate no statistically significant differences (Tukey's test at p<0.05); p-value (*not very significant/ **significant/ ***highly significant).*

In the microvinifications of TNRG and TNSC, the concentration trend decreases instead starting from the polymeric fraction to the monomeric and finally oligomeric one. The differences are significant between the microvinifications of Portuguese (TNPT) and Brazilian (TNRG/TNSC) wines but not significant between the microvinifications of TNRG and TNSC. The monomeric fraction is greater than the oligomeric one: according to what is reported in the paragraph of anthocyanins and pigment contents, (3.3.1) where the polymerization index is higher for Brazilian wines, the data seem consistent. A higher polymerization index could indicate a greater capacity for combination between the monomeric fractions, still free and therefore present in greater quantities than the oligomeric fraction.

The differences between TNPT, TNRG and TNPR microvinifications can be attributed to a number of factors: in general, there is a tendency for higher concentrations of flavanols, in particular proanthocyanidins in the early stages of grape berry development. The high values of these compounds may be related with their metabolization throughout the ripening process. However, during grape maturation, in particular after veraison, several works describe a continual decrease of the proanthocyanidin content until grape harvest (Jordão *et al*.,1998). The decrease after veraison of proanthocyanidins in seeds and skins could be explained by oxidation reactions (Kennedy *et al*., 2000;) and by a reduction of the extractability resulting from the conjugation of proanthocyanidins with other cellular components (Cheynier *et al*., 1997).

However, for other authors the proanthocyanidin decrease is a consequence of different combined factors. Thus, for Valero *et al*., (1989) the proanthocyanidin concentration decrease during grape maturation is only a consequence of the increasing weight of the berries or seeds, while, for Bogs *et al*., (2005) the proanthocyanidin content during grape maturation is a result of a balance between the accumulation of proanthocyanidin through synthesis and decreased extractability.

Several research works establish that vintage (Tounsi *et al*., 2009; Lorrain *et al*., 2011; Kyraleou *et al*., 2016), environmental factors, geographical conditions (Obreque-Slier *et al*., 2010) and also viticultural practices (Jordão *et al*., 1998), are decisive for the grape proanthocyanidin content and also for the evolution of these compounds during grape maturation. According to the results obtained by Fuleki and Ricardo-da-Silva (2003), the content of (1)-catechin, (2)-epicatechin, and nine procyanidins quantified in grape juice were influenced by different factors, namely by decreasing order of importance, by cultivar and vintage. Environmental factors, geographical conditions, and the specific conditions that occur during each vintage could determine the flavonoid pathway and consequently have an impact on proanthocyanidin concentration and distribution (Figure 24).

According to this, studies have shown that proanthocyanidin accumulation reaches a peak close to veraison and decreases towards harvest. This could be ascribed to its extractability rather than a degradation or turnover (De Freitas & Glories, 1999; Kennedy et al., 2001; Ó-Marques et al., 2005). Cohen *et al*., (2008) studied the effect of temperature during the green berry stage and maturation. Proanthocyanidin accumulation was linearly related to the heat summation during the grape development period. Yet, damping of the diurnal temperature by daytime cooling and night-time heating resulted in a reduction in the proanthocyanidins concentration. Downey *et al*., (2004) suggested that shading has no significant effect on the levels of condensed tannins in the skins or seeds of ripe fruit. Cohen *et al*., (2010) found that heating and cooling of berries from 20.5°C by ±8°C altered the initial rates of proanthocyanidin accumulation. Therefore, a greater thermal shock, especially in the Brazilian region of Santa Catarina, could have a negative impact on the synthesis of these compounds; however, the total proanthocyanidin accumulation was not related to the thermal time but is more likely a function of berry development within a particular season (Blancquaert *et al.,* 2018).

In accordance with what was stated in the previous paragraph relating to Tannin power as well, the less temperate but hotter and more humid climatic conditions of the Brazilian

cultivation regions taken into consideration for the development of this study, have disadvantaged the synthesis of these compounds which have been detected in significantly lower concentrations than the Portuguese samples in all three fractions extracted.

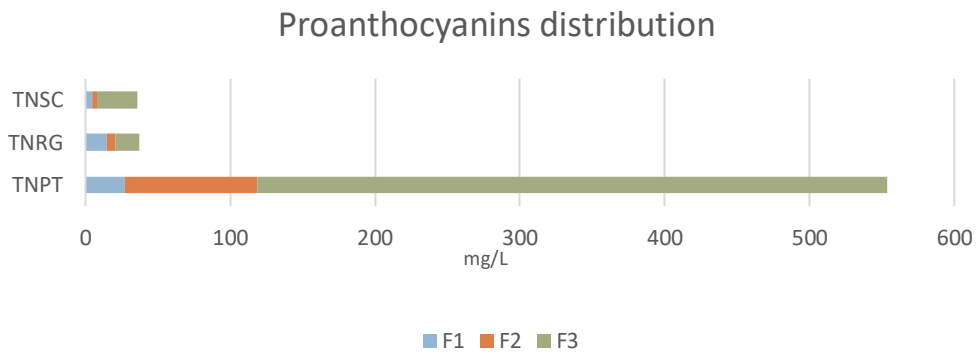


***Figure 25**. Proanthocyanis distribution in Touriga Nacional from Portugal, Rio Grande do Sul and Santa Catarina*

*Legend: **TNPT**: Touriga Nacional Portugal, **TNRG**: Touriga Nacional Rio Grande do Sul, **TNSC:** Touriga Nacional Santa Catarina; **F1**: monomeric fraction, **F2**: oligomeric fraction, **F3**: polymeric fraction.*

3.5 Sensory Analysis

A sensory analysis was conducted for all wines analysed in the present study, for Touriga Nacional wines and for Alvarinho wines grown in Portugal and Brazilian regions. The tasters were asked to evaluate the visual aspect, the aroma and the taste of the wines, in order to then give an overall judgment on the global appreciation. The results elaborated starting from the analysis sheets were elaborated and divided into two spider diagrams for each variety, one relating to the visual and olfactory aspect and one relating to the gustatory aspect.

The figure 25 shows a significant difference in the quality of the aroma, which is greater in PT and SC, compared to that of RG, which is significantly lower; similarly, also the scent of red fruits is perceived with a decreasing intensity from PT to RG. No significant differences emerge for the scent of oak; also, for the floral scent there is a decreasing perception from PT to RG, while the vegetal scent was perceived inversely, with a higher

intensity in RG and lower in SC and PT. The general aromatic balance, including aroma persistence and intensity, does not show significant differences between PT and SC, while it is much lower in RG. From a visual point of view, the color intensity is significantly greater in PT than in the two Brazilian wines SC and RG, between which no significant differences emerged. The clarity was higher in PT, followed by SC and finally in RG.

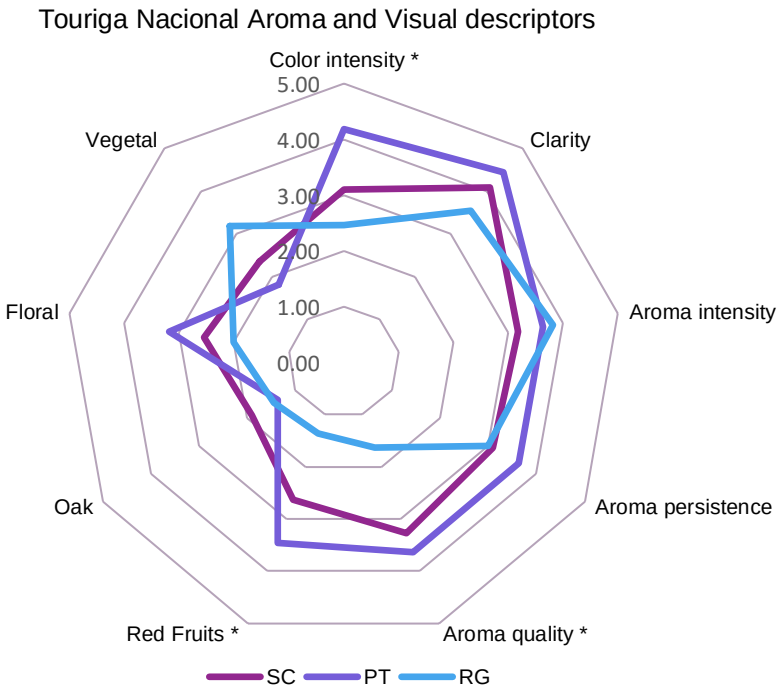


***Figure 26**. Sensory analysis results for Touriga Nacional from Portugal, Rio Grande do Sul and Santa Catarina*

*Legend: **PT**: Touriga Nacional Portugal, **RG**: Touriga Nacional Rio Grande do Sul, **SC:** Touriga Nacional Santa Catarina; an asterisk (*) represents significant differences between the wines (Tukey's test, p<0.05)*

The gustatory examination, shown in figure 26, reported the following values: acidity was perceived more in RG, followed by PT and SC; sweetness is instead greater in SC, decreasing in RG and finally in PT, between which no significant differences emerged. The data show non-significant differences in terms of bitterness in the three wines, resulting slightly higher in SC than the other wines. The tasters found a significantly greater persistence in PT; the difference between SC and RG is not significant. The perception of astringency decreases from PT to RG; the gustatory balance is greater in PT, followed by SC and finally by RG, significantly lower.

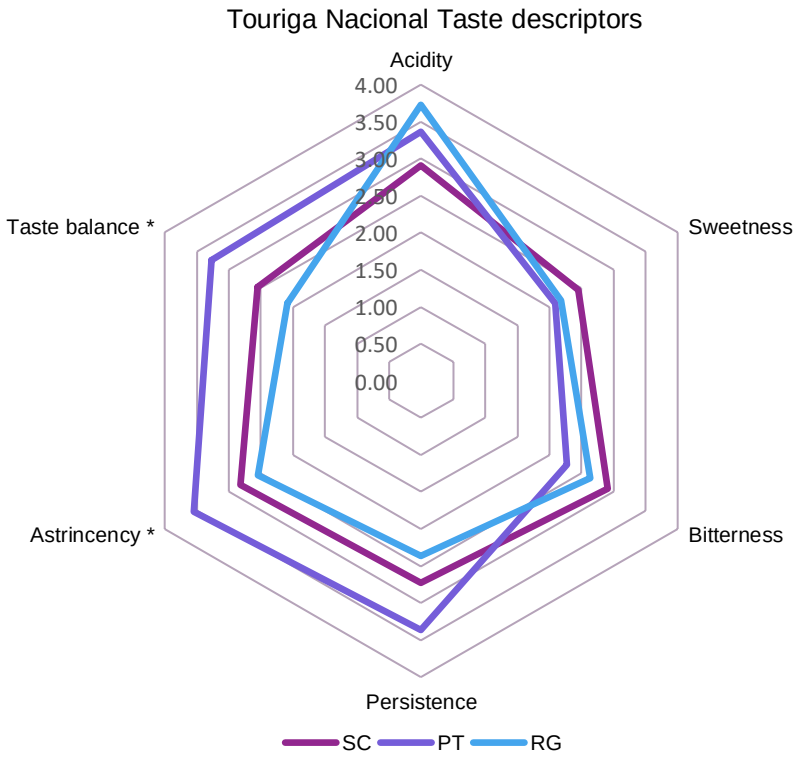


***Figure 27**. Sensory analysis results of Touriga Nacional from Portugal, Rio grande do Sul and Santa Catarina*

*Legend: **PT**: Touriga Nacional Portugal, **RG**: Touriga Nacional Rio Grande do Sul, **SC:** Touriga Nacional Santa Catarina; an asterisk (*) represents significant differences between the wines (Tukey's test, $p<0.05$)*

According to what emerges from these results, and based on the knowledge that phenolic compounds play an essential role in the formation of the flavors and taste of red wines, the highest sensory analysis scores were always attributed to the TNPT, in line with the highest results also obtained in the previous analyses.

The natural conditions (climate and nature of the soil) and the viticultural factors, strongly influence the evolution of the aromatic contents during the vegetative period of the vine. Allen *et al*., (1993) found lower vegetal aromatic contents in Australia in wines derived from grapes whose ripening took place at a higher temperature, while Falcao *et al*., (2007) found higher levels in Brazil in wines derived from grapes grown at higher altitudes. As already stated in chapter 1., (1.1.1 Rio Grande do Sul, 1.1.2 Santa Catarina, 1.2.1 Dão region), each region has its own influencing characteristics: in Rio Grande do Sul the climate is characterized by regular rainfall, interspersed with periods of drought: this variation, its altitude, and predominant rock soil could be the reason of lower aroma balance, due to less regular ripening of the vines (Protas *et al*., 2006).

In Santa Catarina, due to the milder air temperatures in this region, the vegetative and reproductive cycle of the grapevine is more extensive, resulting in slower ripening of the grapes to produce fine quality wines; the low temperatures in the nights cause a hormonal change which leads to a reduction in vegetative growth and the beginning of ripening with an accumulation of sugars, phenols and aroma precursors, guaranteeing a good final aromatic balance. Dão Region has cooler nights and warmer days allow for a slower maturation process, producing a strong aroma and acidity that result in rich and elegant wines; regular ripening and temperate climate increase the good development of aroma (Costa *et al*., 2019).

Sensory analysis was also applied to white wines, PT, RG, PR. The tasters were involved, each of whom was asked to evaluate the colour, aroma and taste on an increasing scale from 1 to 5; each value was associated with an attribute, such as, non-existent, not very intense, quite intense, intense, very intense respectively from 1 to 5.

In the case of Alvarinho wines, compared to Touriga Nacional, the scores attributed to each parameter were similar; the data were collected separately and elaborated in a diagram relating to the aromatic and visual aspect, and one relating to the gustatory aspect. Figure 27 shows how, in general, PT has higher scores than RG and PR, but that the only parameters that showed significant differences were the tropical and citrus scents: the averages calculated on the basis of the scores of these two associated parameters a PT were significantly greater than PR and RG.

The scores attributed to the gustatory aspect did not present any significant difference; as emerges from figure 28, the data overlap in persistence and intensity. Visually, a greater difference is perceived for the body, where PR has a greater value, followed by PT and RG; also, in terms of acidity there is a greater difference between PT/PR and RG, where it was perceived to a lesser extent. Unlike the Touriga Nacional wines, the sensory characteristics of Alvarinho have not been influenced by the environmental and pedoclimatic conditions of the different growing regions.

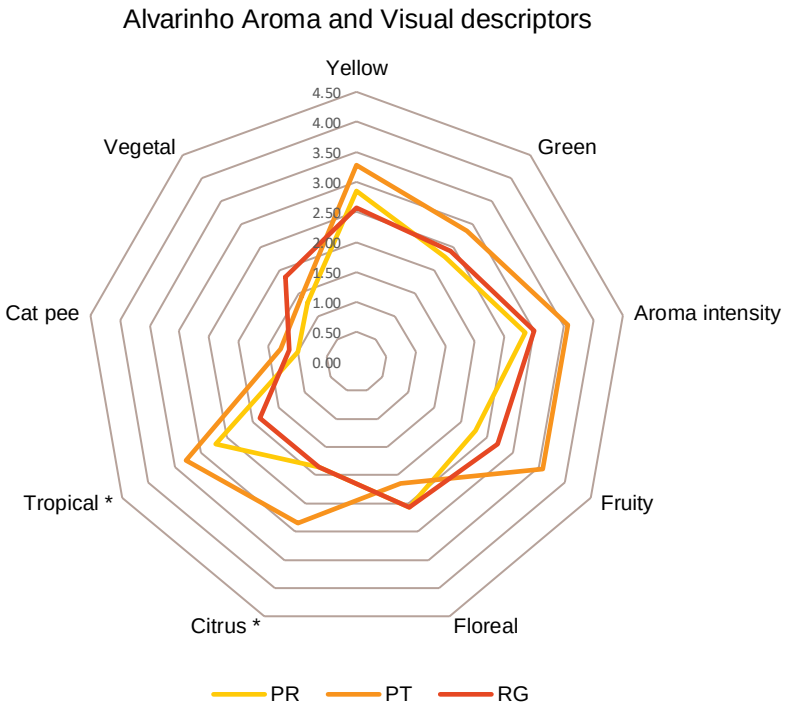


***Figure 28**. Sensory analysis results of Alvarinho from Portugal, Rio Grande do Sul and Paraná State*

*Legend: **PT**: Alvarinho Portugal, **RG**: Alvarinho Rio Grande do Sul, **PR:** Alvarinho Paraná State; an asterisk (*) represents significant differences between the wines (Tukey's test, p<0.05)*

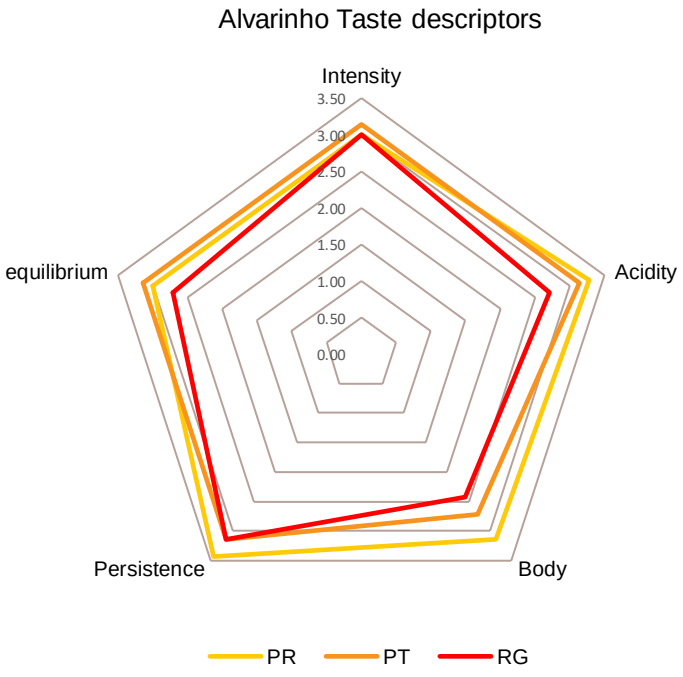


***Figure 29**. Sensory analysis of Alvarinho from Portugal, Rio Grande do Sul and Paraná State*

*Legend: **PT**: Alvarinho Portugal, **RG**: Alvarinho Rio Grande do Sul, **PR:** Alvarinho Paraná State; an asterisk (*) represents significant differences between the wines (Tukey's test, p<0.05)*

Finally, making an analysis of global appreciation, figure 29 shows that Portuguese wines, TNPT and ALPT obtained overall higher scores than Brazilian wines. As already shown in the previous figures, also from this image, it can be seen that significant differences also emerged between the global appreciation of TNSC, a result greater than that shown by the tasters for TNRG. In the case of Alvarinho, there were no significant differences between the overall appreciation for ALPR and ALRG.

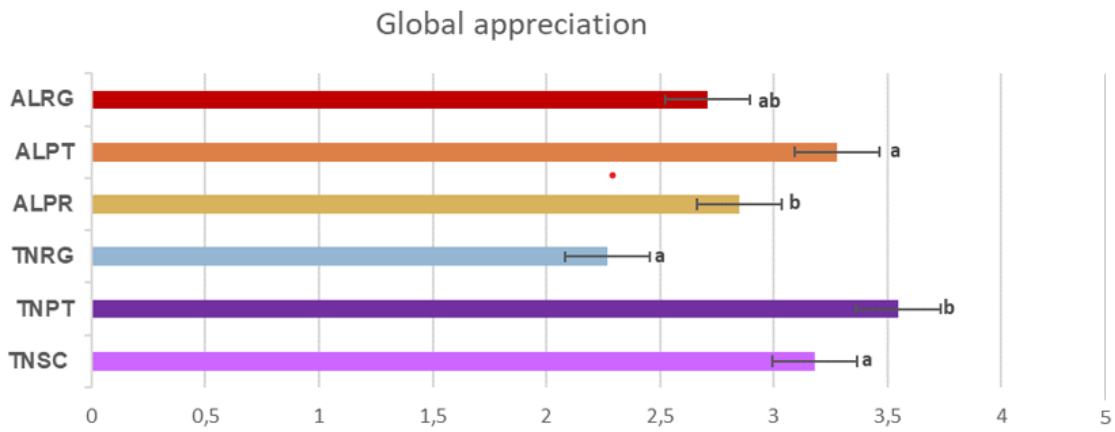


***Figure 30**. Sensory analysis global appreciation results of the wines studied*

*Legend: **TNPT**: Touriga Nacional Portugal, **TNRG**: Touriga Nacional Rio Grande do Sul, **TNSC:** Touriga Nacional Santa Catarina, AL**PT**: Alvarinho Portugal, **ALRG**: Alvarinho Rio Grande do Sul, **ALPR:** Alvarinho Paraná State*

4. Conclusions

In recent decades, numerous studies have been carried out on the identification and characterization of polyphenolic compounds in the wine sector. Furthermore, large improvements have been obtained in the investigation of the change of grape compounds during the grape maturation and the relationship between their content in different bunch fraction and the diverse environmental factors that determine the proanthocyanins accumulation during the entire period of maturation.

Producing wines in hot tropical climatic conditions requires particular techniques for the quality of the grapes and specific harvesting techniques; it is necessary to pay attention to the type of treatment of the selected must and crushed grapes, and to take particular care in the pressing and fermentation systems, in temperature control, in aging and finally in stabilizing the wines. On the basis of all the scientific studies previously examined regarding the varietal adaptability of native vines in non- originating countries, the following study set out to give analytical results to the hypotheses that arose on what is the adaptability response of the same varieties grown in Portugal and in Brazil.

The research was done by carrying out laboratory analyses at the Instituto Superior de Agronomia, on samples of 2022 microvinifications of Alvarinho and Touriga Nacional provided by the Polytechnic Institute of Viseu. The development of the thesis was based on a recurring question during the analyses: is it possible that the polyphenolic composition of the same variety differs according to the country of cultivation and its different pedo-climatic conditions? For which parameters is it possible to highlight this gap? How does the terroir affect these compounds? And how do these compounds influence the qualitative characterization of wines in one country compared to another? Following the processing of the data obtained, it is possible to state that there is a minimum divergence between the samples of Alvarinho of Vinhos Verdes and those of Rio Grande do Sul and Paranà State; on the other hand, a notable difference emerged in terms of polyphenolic compounds between the Touriga Nacional wine samples grown in the Dão Region, compared to those of Touriga Nacional grown in Rio Grande do Sul and Santa Catarina.

The phenolic compounds of white berried varieties are present in smaller quantities than the concentration of these found in red berried varieties: total phenols, non-flavonoids, flavonoids and chromatic parameters (a*/b*/L*/C */h*/H*) that were taken into consideration for the study of Alvarinho, did not show significant results and were not

influenced by the different environmental conditions of Portugal and Brazil. Touriga Nacional was subjected to a more in- depth compound analysis, also considering tannin power, determination and fractionation of proanthocyanidins, copygmentation, anthocyanins and colouring pigments. The study shows a constant higher concentration of these compounds in the wines of Touriga Nacional grown in Portuguese territory.

According to some scientific research previously reported on the climatic difference between the two countries that were the subject of this study, it is possible to state that the results obtained are in line: higher daytime temperatures, and greater humidity, decrease the accumulation of anthocyanins, suggesting a strong effect of temperature on the biosynthesis of phenolic compounds and/or degradation of anthocyanins. The final concentration of these compounds therefore depends on the balance between their synthesis and degradation, the latter stimulated by high temperatures. The different concentrations also influenced the overall appreciation during tasting, proving that these molecules also have a significant influence on the quality of the wine.

However, it is not possible to attribute these differences found only to the climatic conditions in which these cultivations took place: the stability of these compounds and their more or less high concentration may also have been determined by a different winemaking process, and depend on various factors such as the type of molecule, the concentration of the solutions, the pH, the maceration temperature, the oxidation state, the light and the nature of the solvent. Although the results obtained may be the answer that the phenolic composition of the grapes is influenced by the surrounding environment, it is good to leave room for the hypothesis that some data are also the result of adopting a different oenological approach. Within this experimental study, however, no other chemical parameters or the winemaking techniques adopted were analysed but was limited to a purely phenolic analysis.

In conclusion of this, it is hoped that the results that will, constitute a contribution to the current oenological scientific knowledge inherent to the crops developed in the "new world"; moreover, it is possible to affirm that this experimentation was also carried out with the aim of collecting data that could be an incentive for other future studies.

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ANNEXES

Annex I. Alvarinho Total Phenols, Non-Flavonoids, Flavonoids results: ALPT (Alvarinho Portugal), ALRG (Alvarinho Rio Grande do Sul), ALPR (Alvarinho Paranà State); *(1st, 2nd, 3rd)* : first, second and third microvinification; P(>F) = p-value (*not very significant/ **significant/ ***highly significant); Microvinification = Micr.Vin; Standard Deviation = Stand.Dev.

Wine	Micr.Vin	Sample	Total Phenols		Non-Flavonoids		Flavonoids	
			Average	Stand.Dev	Average	Stand.Dev	Average	Stand.Dev
ALPT	1st	1	240,570	1,271	101,215	1,271	139,555	1,271
ALPT	1st	2						
ALPT	2nd	1	240,303	2,506	101,227	2,506	139,076	2,506
ALPT	2nd	2						
ALPT	3rd	1	238,012	1,987	94,455	1,987	143,557	1,987
ALPT	3rd	2						
ALRG	1st	1	304,531	0,537	115,772	0,537	188,759	0,537
ALRG	1st	2						
ALRG	2nd	1	303,645	3,939	114,438	3,939	189,204	3,939
ALRG	2nd	2						
ALRG	3rd	1	303,645	1,790	114,793	1,790	188,852	1,790
ALRG	3rd	2						
ALPR	1st	1	309,619	1,469	111,418	1,469	198,201	1,469
ALPR	1st	2						
ALPR	2nd	1	310,139	3,598	116,240	3,598	193,899	3,598
ALPR	2nd	2						
ALPR	3rd	1	305,380	0,412	112,620	0,412	192,760	0,412
ALPR	3rd	2						
General Means	ALPT		239,628b		98,965b		140,729b	
	ALRG		303,940a		115,001a		188,938ab	
	ALPR		308,379a		113,426a		194,953a	
Pr(>F)			1618.1 < 2.2e-16 ***		4.711e-08 ***		1.09e-15 ***	

Annex II. Touriga Nacional Total Phenols, Non-Flavonoids, Flavonoids results: TNPT (Touriga Nacional Portugal), TNRG (Touriga Nacional Rio Grande do Sul), TNSC (Touriga Nacional Santa Catarina); *(1st, 2nd, 3rd)* : first, second and third microvinification; P(>F) = p-value (*not very significant/ **significant/ ***highly significant); Microvinification = Micr.Vin; Standard Deviation = Stand.Dev.

Wine	Micr.Vin	Sample	Total Phenols		Non-Flavonoids		Flavonoids	
			Average	Stand.Dev	Average	Stand.Dev	Average	Stand.Dev
TNPT	1st	1	2203,130	41,889	128,175	8,379	2075,076	50,099
TNPT	1st	2						
TNPT	2nd	1	2247,695	22,196	109,873	1,218	2137,816	23,413
TNPT	2nd	2						
TNPT	3rd	1	2242,120	35,440	114,580	1,895	2127,541	37,337
TNPT	3rd	2						
TNRG	1st	1	582,759	8,055	217,429	1,771	365,3305	9,825
TNRG	1st	2						
TNRG	3rd	1	576,557	22,556	220,822	0,376	355,735	22,932
TNRG	3rd	2						
TNSC	2nd	1	855,037	20,765	213,822	4,511	641,213	25,279
TNSC	2nd	2						
TNSC	3rd	1	829,848	10,562	212,696	0,734	617,152	11,296
TNSC	3rd	2						
General Means	TNPT		2230,981c		117,542b		2113,477a	
	TNRG		579,658b		219,125a		360,532c	
	TNSC		842,442a		142,172b		629,182b	
Pr(>F)			5793.2 < 2.2e-16 ***		6.276e-11 ***		4546.2 < 2.2e-16 ***	

Annex III. Alvarinho CIE/Lab results: ALPT (Alvarinho Portugal), ALRG (Alvarinho Rio Grande do Sul), ALPR (Alvarinho Paranà State); P(>F) = p-value (*not very significant/ **significant/ ***highly significant); *(1st, 2nd, 3rd)* : first, second and third microvinification; Microvinification = Mv, Sample =S; Average = Av, Standard Deviation = St. D.; Clarity = L*, Red-green colour = a*, Yellow – blue colour = b*, Chroma = C*, Hue = H*, H*correction = H* C.

Wine	Mv	S	L*		a*		b*		C*		H*		H*C	
			Av.	St.D	Av.	St.D	Av.	St.D	Av.	St.D	Av.	St.D	Av.	St.D
ALPT	1st	1	93,29	0,303	-0,0292	0,039	10,77	0,100	10,77	0,100	-89,843	0,206	270,15	0,206
ALPT	1st	2												
ALPT	2nd	1	96,59	0,505	-0,6720	0,086	8,946	0,123	8,970	0,127	-85,704	0,489	274,29	0,489
ALPT	2nd	2												
ALPT	3rd	1	95,76	0,163	-0,2693	0,034	9,735	0,059	9,738	0,057	-88,414	0,209	271,58	0,209
ALPT	3rd	2												
ALRG	1st	1	95,00	0,004	-1,0343	0,015	17,18	0,033	17,21	0,034	-86,556	0,045	273,44	0,045
ALRG	1st	2												
ALRG	2nd	1	94,62	0,095	-1,0630	0,014	8,051	0,020	17,19	0,020	-86,456	0,049	273,54	0,049
ALRG	2nd	2												
ALRG	3rd	1	95,56	0,012	-1,1352	0,025	16,50	0,038	16,55	0,040	-86,064	0,076	273,93	0,076
ALRG	3rd	2												
ALPR	1st	1	96,87	0,034	-1,8672	0,020	16,53	0,030	16,64	0,028	-83,556	0,081	276,44	0,081
ALPR	1st	2												
ALPR	2nd	1	96,04	0,075	-1,6800	0,008	16,75	0,016	16,86	0,027	-84,271	0,033	275,73	0,033
ALPR	2nd	2												
ALPR	3rd	1	97,29	0,321	-2,2627	0,007	15,84	0,080	16,00	0,078	-81,871	0,064	278,13	0,064
ALPR	3rd	2												
General Means	ALPT		95,21 b		-0,3235a		9,817 c		9,826 b		-87,987b		272,00b	
	ALRG		95,06 b		-1,0775b		13,91 b		16,98 a		-86,358b		273,63b	
	ALPR		96,73 a		-1,9366c		16,37 a		16,50 a		-83,232a		276,76a	
P(>F)			0.01903 *		1.796e-08 ***		1.088 e-12 ***		6.976 e-13 ***		3.876e-05 ***		3.876e-05 ***	

Annex IV. Touriga Nacional CIE/Lab results: TNPT (Touriga Nacional Portugal), TNRG (Touriga Nacional Rio Grande do Sul), TNSC (Touriga Nacional Santa Catarina); P(>F) = p-value (*not very significant/ **significant/ ***highly significant); *(1st, 2nd, 3rd)* : first, second and third microvinification; Microvinification = Mv, Sample = S; Average = Av, Standard Deviation = St. Dev.; Clarity = L*, Red- green colour = a*, Yellow – blue colour = b*, Chroma = C*, Hue = H*, H*correction = H* C.

Wine	Mv	S	L*		a*		b*		C*		H*		H*C	
			Av.	St.D	Av.	St.D	Av.	St.D	Av.	St.D	Av.	St.D	Av.	St.D
TNPT	1st	1	65,23	0,186	34,02	0,273	0,908	0,080	34,03	0,271	1,530	0,148	1,530	0,148
TNPT	1st	2												
TNPT	2nd	1	60,17	0,019	39,96	0,076	0,008	0,488	39,97	0,076	-0,495	0,016	359,50	0,016
TNPT	2nd	2												
TNPT	3rd	1	65,75	0,284	33,37	0,172	1,083	0,046	33,38	0,171	1,859	0,088	1,845	0,107
TNPT	3rd	2												
TNRG	1st	1	85,27	0,211	15,53	0,228	-1,022	0,017	15,41	0,228	-3,799	0,002	356,20	0,002
TNRG	1st	2												
TNRG	3rd	1	85,29	0,108	15,67	0,020	-0,829	0,033	15,69	0,022	-3,027	0,115	356,97	0,115
TNRG	3rd	2												
TNSC	2nd	1	80,36	0,396	16,74	0,027	0,517	0,182	16,74	0,022	1,770	0,626	1,770	0,626
TNSC	2nd	2												
TNSC	3rd	1	80,20	0,031	16,92	0,028	0,043	0,041	18,42	2,092	0,146	0,139	0,146	0,139
TNSC	3rd	2												
General Means	TNPT		63,71c		35,78 a		0,666 a		35,79 a		0,964 a		120,95b	
	TNRG		85,28 a		15,60 b		-0,925 b		15,55 b		-3,413 b		356,58a	
	TNPR		80,28 b		16,83 b		0,28a		17,58 b		0,958 a		0,958c	
P(>F)			3.311 e-09 ***		1.7e-08 ***		0.3124 ***		3.899 e-08 ***		3.779 e-05 ***		0.005823 **	

Annex V. Touriga Nacional copygmentation results: TNPT (Touriga Nacional Portugal), TNRG (Touriga Nacional Rio Grande do Sul), TNSC (Touriga Nacional Santa Catarina); *(1st, 2nd, 3rd)* : first, second and third microvinification; Microvinification = Micr.Vin; P(>F) = p-value (*not very significant/ **significant/ ***highly significant); Copygmentation value multiply per 20,03 = CC*20,03, Standard Deviation = Standard Dev.

Wine	Micr.Vin	Sample	CC*20,03 (mg malvidin 3-O-glucosid L-1)	
			Average	Stand.Dev
TNPT	1st	1	205,251	0,197
TNPT	1st	2		
TNPT	2nd	1	201,690	4,033
TNPT	2nd	2		
TNPT	3rd	1	196,940	6,187
TNPT	3rd	2		
TNRG	1st	1	42,691	0,776
TNRG	1st	2		
TNRG	3rd	1	45,269	0,287
TNRG	3rd	2		
TNSC	2nd	1	55,599	0,313
TNSC	2nd	2		
TNSC	3rd	1	55,744	0,23
TNSC	3rd	2		
General Means	TNPT		201,29a	
	TNRG		43,98b	
	TNSC		55,67b	
P(>F)			5.218e-16 ***	

Annex VI. Touriga Nacional colour components results: TNPT (Touriga Nacional Portugal), TNRG (Touriga Nacional Rio Grande do Sul), TNSC (Touriga Nacional Santa Catarina); *(1st, 2nd, 3rd)* : first, second and third microvinification; P(>F) = p-value (*not very significant/ **significant/ ***highly significant); Microvinification = Mv, Colour Intensity = C.I., Average = Av, Standard Deviation = St. Dev, Tonality = TO, Total Antocyanins = T.A., Degree of Ionizzation = I.D., Ionized Antocyanins = I.A., Polymerization index = P.I., Total pigments = T.P., Polymerized pigments = P.P.

W	Mv	S	C.I		TO		T.A.		I.D.		I.A.	
			Av	St.D	Av	St.D	Av	St.D	Av	St,D	Av	St.D
TNPT	1st	1	11,872	0,536	0,726	0,033	304,265	3,585	16,780	3,150	50,994	8,958
TNPT	1st	2										
TNPT	2nd	1	14,148	0,04	0,647	0,007	261,930	5,303	29,560	2,135	77,382	4,009
TNPT	2nd	2										
TNPT	3rd	1	11,698	0,139	0,716	0,004	481,195	45,511	10,820	0,544	51,955	2,298
TNPT	3rd	2										
TNRG	1st	1	3,908	0,162	0,558	0,006	8,830	1,2300	61,960	1,909	5,460	0,594
TNRG	1st	2										
TNRG	3rd	1	4,333	0,011	0,578	0,004	9,075	0,728	51,010	8,450	4,598	0,395
TNRG	3rd	2										
TNSC	2nd	1	5,853	0,145	0,703	0,013	5,674	1,519	89,105	8,026	4,994	0,898
TNSC	2nd	2										
TNSC	3rd	1	5,807	0,033	0,688	0,003	5,900	0	73,255	15,875	4,320	0,933
TNSC	3rd	2										
General Means	TNPT		12,57a		0,696a		349,13a		19,05b		60,12a	
	TNRG		4,120b		0,568b		8,950b		56,48ab		5,029b	
	TNSC		5,825b		0,695a		5,787b		81,18a		4,657b	

P.I.		T.P.		P.P	
Av	St.D	Av	St.D	Av	St.D
16,612	0,018	21,03	0,251	3,49	0,045
19,440	0,042	19,37	0,371	3,77	0,064
11,458	0,973	29,72	2,221	3,40	0,033
52,555	1,237	3,57	0,099	1,88	0,096
53,075	0,375	3,93	0,100	2,09	0,038
56,335	1,068	4,63	0,067	2,61	0,091
56,240	0,042	4,71	0,107	2,65	0,058
15,836b		23,373a		3,55a	
52,815a		3,75b		1,98b	
56,287a		4,67b		2,63ab	
4.786e-11 ***		2.084e-06 ***		9.487e-09 ***	

Annex VII. Touriga Nacional absorbance results previously used in order to obtain the colour components showed in Table 5 (Somers and Evans *et al*., 2001); *(1st, 2nd, 3rd)* : first, second and third microvinification; Absorbance measured at 420nm; Absorbance measured at 520nm; Absorbance measured at 620nm; Absorbance measured at 520nm with SO2 addiction; Absorbance measured at 520nm with HCl interference.

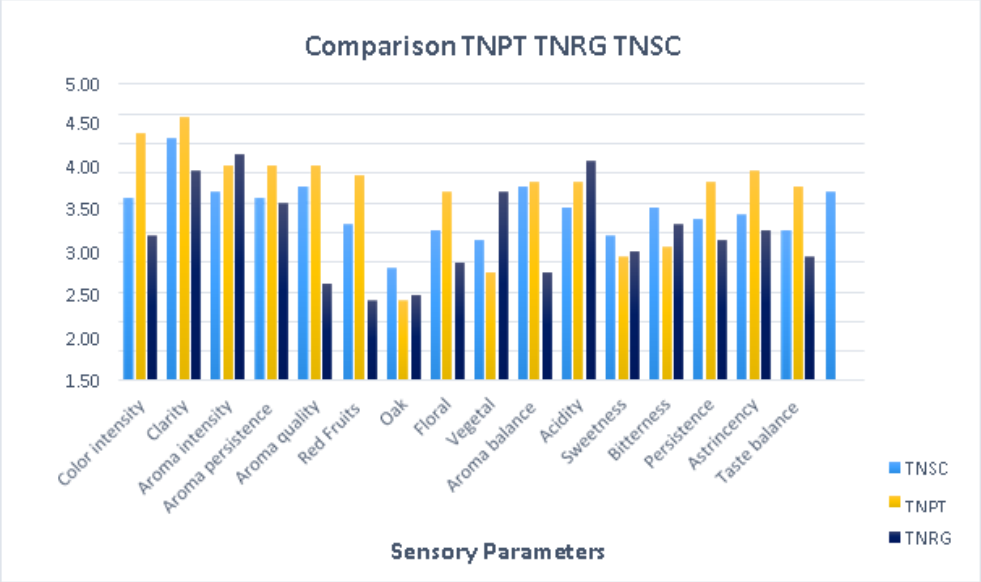
TNPT	1st	1	4,446	6,329	1,476	3,526	20,8565
TNPT	1st	2	4,315	5,759	1,419	3,721	21,2100
TNPT	2nd	1	4,968	7,732	1,419	3,812	19,1092
TNPT	2nd	2	4,916	7,539	1,721	3,373	19,6344
TNPT	3rd	1	4,318	6,052	1,426	3,419	31,2898
TNPT	3rd	2	4,264	5,935	1,399	1,807	28,1487
TNRG	1st	1	1,164	2,101	0,528	1,943	3,4960
TNRG	1st	2	1,235	2,195	0,592	2,112	3,6360
TNRG	3rd	1	1,355	2,328	0,659	2,058	3,9990
TNRG	3rd	2	1,324	2,302	0,699	2,672	3,8582
TNSC	2nd	1	2,060	2,890	1,005	2,549	4,6800
TNSC	2nd	2	1,964	2,830	0,956	2,609	4,5854
TNSC	3rd	1	1,962	2,859	0,962	2,691	4,6359
TNSC	3rd	2	1,985	2,874	0,971	3,526	4,7874

Annex VIII. Touriga Nacional Tannin power results: TNPT (Touriga Nacional Portugal), TNRG (Touriga Nacional Rio Grande do Sul), TNSC (Touriga Nacional Santa Catarina); *(1st, 2nd, 3rd)* : first, second and third microvinification; Microvinfication = Micr.Vin; P(>F) = p-value (*not very significant/ **significant/ ***highly significant); Standard Deviation = Standard Dev.

Wine	Micr.Vin	Sample	Tannin power NTU/Ml-1	
			Average	Stand.Dev
TNPT	1st	1	531,100	12,215
TNPT	1st	2		
TNPT	2nd	1	587,344	12,772
TNPT	2nd	2		
TNPT	3rd	1	545,681	52,193
TNPT	3rd	2		
TNRG	1st	1	77,281	1,458
TNRG	1st	2		
TNRG	3rd	1	84,462	0,389
TNRG	3rd	2		
TNSC	2nd	1	290,140	10,550
TNSC	2nd	2		
TNSC	3rd	1	261,385	45,064
TNSC	3rd	2		
General Means	TNPT		554,708a	
	TNRG		80,87c	
	TNSC		275,76b	
P(>F)			1.611e-10 ***	

Annex IX. Touriga Nacional proanthocyanidins separation results based on their polymerization grade: TNPT (Touriga Nacional Portugal), TNRG (Touriga Nacional Rio Grande do Sul), TNSC (Touriga Nacional Santa Catarina); *(1st, 2nd, 3rd)* : first, second and third microvinification; P(>F) = p-value (*not very significant/ **significant/ ***highly significant); Microvinification = Micr.Vin; Standard Deviation = St. Dev, Monomeric fraction = F1, Oligomeric fraction = F2, Polymeric fraction = F3.

Wine	Micr.Vin	F1		F2		F3	
		Average	St.Dev	Average	St.Dev	Average	St.Dev
TNPT	1st	27,407a	0,349	91,304a	6,149	435,13a	118,483
TNPT	2nd						
TNRG	1st	14,412b	9,034	5,975b	0,771	16,892b	0,955
TNRG	3rd						
TNSC	2nd	4,935c	0,870	2,715b	0,771	28,378b	1,911
TNSC	3rd						
P(>F)		0.05207		0.0002388 ***		0.01406 *	



Annex X. The summarized mean and standard deviation values, relating to each attribute, for each type of wine analysed: Santa Catarina Touriga Nacional (SC), Portugal Touriga Nacional (PT), Rio Grande do Sul Touriga Nacional (RG).

Annex XI. The summarized mean and standard deviation values, relating to each attribute, for each type of wine analysed: Santa Catarina Touriga Nacional (SC), Portugal Touriga Nacional (PT) and Rio Grande do Sul Touriga Nacional (RG).

Wines	Visual aspect		Aroma								Taste						Global appreciation
	Color intensity	Clarity	Aroma intensity	Aroma persistence	Aroma quality	Red Fruits	Oak	Floral	Vegetal	Aroma balance	Acidity	Sweetness	Bitterness	Persistence	Astrincency	Taste balance	
SC	3,09	4,09	3,18	3,09	3,27	2,64	1,91	2,55	2,36	3,27	2,91	2,45	2,91	2,73	2,82	2,55	3,18
	0,302	0,302	0,405	0,539	0,647	0,505	0,302	0,522	0,505	0,647	0,539	0,522	0,701	0,467	0,603	0,688	0,405
PT	4,18	4,45	3,64	3,64	3,64	3,45	1,36	3,18	1,82	3,36	3,36	2,09	2,27	3,36	3,55	3,27	3,55
	0,405	0,522	0,505	0,505	0,505	0,522	0,505	0,405	0,405	0,505	0,505	0,701	0,647	0,505	0,522	0,647	0,522
RG	2,45	3,55	3,82	3,00	1,64	1,36	1,45	2,00	3,18	1,82	3,73	2,18	2,64	2,36	2,55	2,09	2,27
	0,820	0,522	0,603	1,095	0,674	0,505	0,688	0,775	0,874	0,751	0,467	0,874	0,505	0,674	0,522	0,539	0,786

Ficha de Prova – Vinhos Tintos

Provador: _____ Data: _____

Aspeto, Aroma e Sabor

Escala: **1** (menos intenso) a **5** (mais intenso)

Apreciação Global

Escala: **1** (Medíocre); **2** (Satisfatório); **3** (Bom); **4** (Muito Bom); **5** (Excelente)

Amostras de vinho									
Aspeto									
Intensidade da Cor									
Limpidez									
Aroma									
Intensidade									
Persistência do Aroma									
Qualidade do aroma									
Frutos vermelhos									
Madeira									
Floral									
Vegetal									
Equilíbrio									
Sabor									
Acidez									
Doce									
Amargo									
Persistência									
Adstringência									
Equilíbrio									
Apreciação Global									

Observações:

_____

_____

_____

Annex XII. Common sheet used during Touriga Nacional sensory analysis

Annex XIII. Common sheet used during Alvarinho sensory analysis

Ficha de Prova de Vinho Brancos

Nome: LP Data 16/06/2023 Sessão: 1 - Vinhos Brancos

Prove os vinhos na ordem apresentada e classifique os diferentes atributos utilizando as seguintes escalas:

Para Cor, Aroma e Gosto: **1.** Inexistente **2.** Pouco Intenso(a) **3.** Medianamente Intenso(a) **4.** Intenso(a) **5.** Muito Intenso(a)

Para Equilíbrio (Aroma e Gosto) e Apreciação Global: **1.** Medíocre **2.** Satisfatório **3.** Bom **4.** Muito Bom **5.** Excelente

		VINHOS / CÓDIGOS															
		ALB	ALP	ALPR													
CÔR	AMARELO	2	4	3													
	VERDE	3	1	2													
AROMA	INTENSIDADE	4	3	4													
	FRUTADO	3	4	1													
	FLORAL	2	2	2													
	TORANJA	1	2	2													
	MARACUJÁ	1	4	3													
	CHICHI DE GATO	1	1	1													
	VEGETAL	1	1	1													
	EQUILÍBRIO	3	4	2													
GOSTO	INTENSIDADE	3	4	3													
	ACIDEZ	3	4	4													
	VOLUME	2	2	3													
	PERSISTÊNCIA	4	4	4													
	EQUILÍBRIO	3	4	3													
APRECIAÇÃO GLOBAL		3	4	3													

Observações:

Annex XIV. Individual anthocyanins means concentrations results for each microvinification sample of wine analyzed; Touriga Nacional Portugal first, second and third microvinfication (TNPT1, TNPT2, TNPT3), Touriga Nacional Rio Grande do Sul first and third microvinfication (TNRG1, TNRG3) and Touriga Nacional Santa Catarina second and third microvinification.

	Delphinidin-3-glucoside			Petunidin-3-glucoside			Peonidin-3-glucoside			Malvidin-3-glucoside			Sum
Vial	Areas	Conc.	Conc. méd	Areas	Conc.	Conc. méd	Areas	Conc.	Conc. méd	Areas	Conc.	Conc. méd	monoglu
TNPT1	16,62	14,03	13,36	66,57	60,62	60,60	35,36	31,51	31,80	79,99	73,14	73,97	179,72
	15,19	12,69		66,51	60,57		35,98	32,09		81,76	74,79		
TNPT2	12,78	10,45	10,96	65,21	59,35	59,63	34,21	30,44	30,33	55,67	50,46	50,29	151,22
	13,89	11,48		65,81	59,91		33,99	30,23		55,32	50,13		
TNPT3	24,78	21,64	21,74	61,34	55,74	56,06	32,45	28,79	28,90	202,52	187,44	186,27	292,96
	24,99	21,84		62,01	56,37		32,67	29,00		200,00	185,09		
TNRG1	0,00	0,00	0,00	2,30	0,67	0,53	0,00	0,00	0,00	6,00	4,12	5,04	5,58
	0,00	0,00		2,01	0,40		0,00	0,00		7,98	5,97		
TNRG3	0,00	0,00	0,00	2,78	1,12	1,08	0,00	0,00	0,00	5,99	4,11	4,17	5,25
	0,00	0,00		2,69	1,03		0,00	0,00		6,12	4,23		
TNSC2	0,00	0,00	0,00	1,98	0,37	0,23	0,00	0,00	0,00	4,56	2,78	2,67	2,90
	0,00	0,00		1,68	0,09		0,00	0,00		4,32	2,55		
TNSC3	0,00	0,00	0,00	1,87	0,27	0,19	0,00	0,00	0,00	4,10	2,35	2,40	2,58
	0,00	0,00		1,70	0,11		0,00	0,00		4,20	2,44		

Delphinidin-3-acetylglucoside			Petunidin-3-acetylglucoside			Peonidin-3-acetylglucoside			Malvidin-3-acetylglucoside			Sum
Areas	Conc.	Conc. méd	Areas	Conc.	Conc. méd	Areas	Conc.	Conc. méd	Areas	Conc.	Conc. méd	acetylate
6,47	4,56	4,55	13,21	10,85	10,91	5,93	4,06	4,08	61,64	56,02	55,42	74,96
6,46	4,55		13,34	10,97		5,98	4,10		60,34	54,81		
5,67	3,81	3,70	12,45	10,14	9,92	5,10	3,28	3,23	50,43	45,57	45,55	62,41
5,43	3,59		11,99	9,71		4,99	3,18		50,40	45,54		
7,23	5,27	5,21	10,45	8,27	8,38	3,68	1,96	2,06	70,50	64,29	63,79	79,43
7,10	5,15		10,68	8,49		3,89	2,15		69,43	63,29		
0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	2,10	0,48	0,43	0,43
0,00	0,00		0,00	0,00		0,00	0,00		1,99	0,38		
0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	2,45	0,81	0,69	0,69
0,00	0,00		0,00	0,00		0,00	0,00		2,20	0,58		
0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,78	0,18	0,13	0,13
0,00	0,00		0,00	0,00		0,00	0,00		1,67	0,08		
0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,65	0,06	0,09	0,09
0,00	0,00		0,00	0,00		0,00	0,00		1,70	0,11		

Malvidin-3-*p*-coumaroylglucoside			Sum coumaroyl derivates (mg/L)
Areas	Conc.	Conc. média (mg/L)	
38,72	34,64	34,55	34,55
38,51	34,45		
35,67	31,80	31,33	31,33
34,67	30,87		
32,56	28,90	28,58	28,58
31,89	28,27		
4,20	2,44	2,40	2,40
4,10	2,35		
4,25	2,49	2,53	2,53
4,34	2,57		
3,98	2,24	2,29	2,29
4,10	2,35		
4,98	3,17	3,12	3,12
4,87	3,07		